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Control of Red Rust Thrips on Bananas in Honduras[1]

JOHN L. NICKEL,[2] *Research Department, Standard Fruit Co., La Ceiba, Honduras*

ABSTRACT

Studies conducted on the control of the red rust thrips, *Chaetanophothrips orchidii* (Moulton) on Giant Cavendish bananas in the Aguan Valley of Honduras indicated that excellent control was obtained with two applications of 1 quart per acre of dieldrin emulsifiable concentrate (1.5 lbs. active ingredient per gallon) 14 days apart, incorporated in the normal helicopter applications of spray oil used for leaf spot control. This control lasted at least 7 months and produced no noticeable adverse side effects. Studies on the relationship between numbers of thrips and incidence of red rust on near-harvest fruit indicated that a population of approximately one thrips for every two fingers of bananas was associated with a 50% incidence of red rust.

Red rust is a peel blemish of bananas caused by the feeding and oviposition of the red rust thrips, *Chaetanophothrips orchidii* (Moulton). The red rust thrips has been taken from tomato, citrus, and a number of weed species (Simmonds 1959) but is chiefly a pest of bananas. Hood (1954) reported that this species had been seen from Australia, Fiji, Panama, Trinidad, Brazil, Honduras, Costa Rica and Florida. He indicated that two structurally different forms exist, one parthenogenetic, the other not. Roberts (1958) reported that only the parthenogenetic form is found on the north coast of Honduras.

Eggs are inserted into the peel early in the development of the bananas. Feeding of young and adults continues on the fruit until harvest. The resultant blemish is a combination of the small, brown, oviposition spots and the superficial russetting caused by the feeding. The injured areas are rendered less elastic resulting in epidermal cracking as the fruit enlarges. Damage is chiefly restricted to areas surrounding points of contact of the fingers. Typical bananas with mild to severe red rust are pictured in figure 1.

Roberts (1958) reviewed the history of control practices from protection by bagging to insecticidal treatment with DDT, lindane, and dieldrin. He reported excellent control with dieldrin, both as granules, applied by air, and as a high volume fruit spray. The dieldrin granules gave long-lasting control but were associated with sub-

[1] Accepted for publication March 27, 1961.
[2] Now with U. S. Operations Mission to Cambodia.

FIG. 1.—Bananas affected to various degrees with banana red rust.

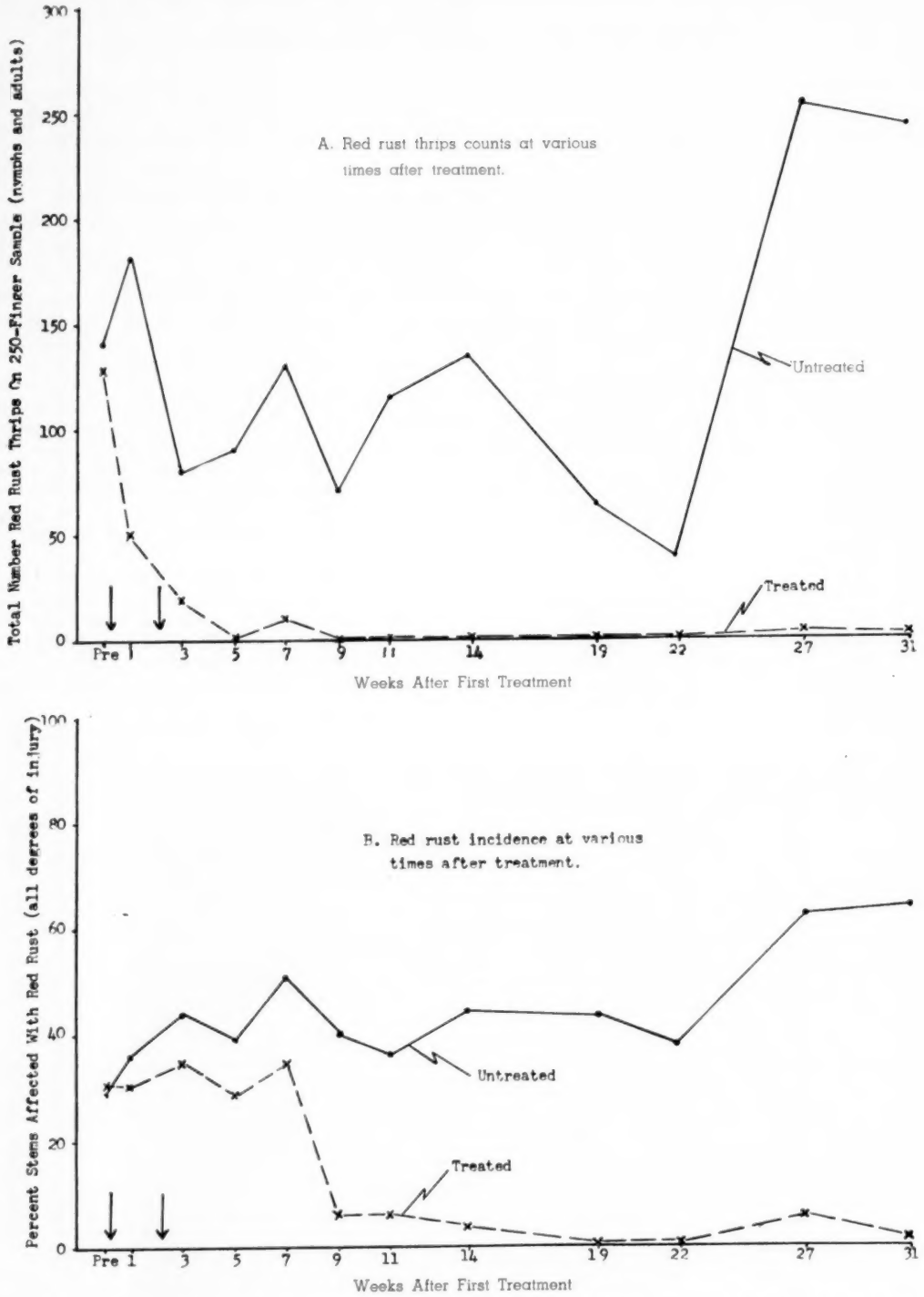

FIG. 2.—Counts of red rust thrips and red rust incidence in treated and untreated areas over a period of 7 months after first treatment. Dieldrin applied in spray oil by air the day after pre-treatment counts and once more 14 days later (arrows).

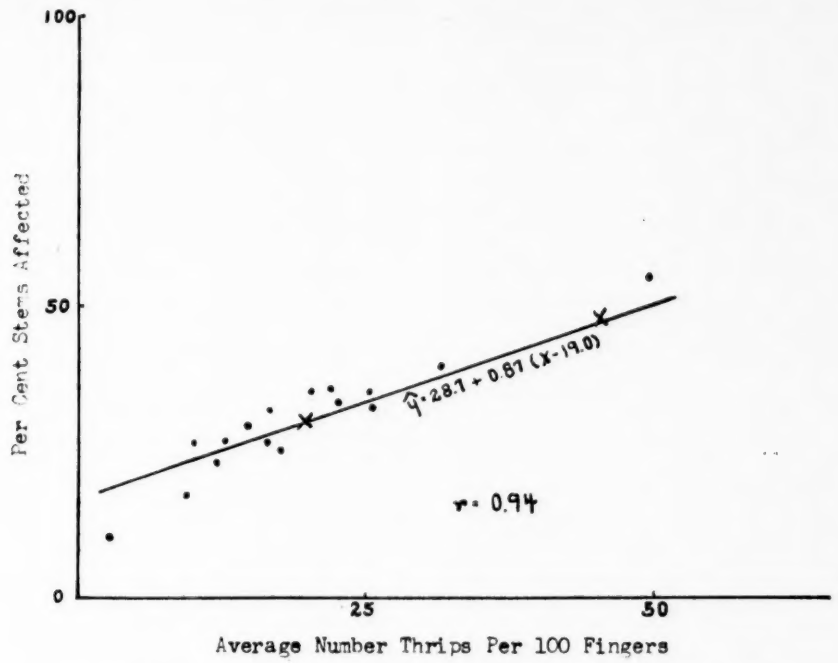

FIG. 3.—Relationship of numbers of thrips to percentages of stems affected with red rust.

sequent outbreaks of fruit-scarring caterpillars and stalk borers. The DDT treatments were associated with outbreaks of red scale. Bullock (1960) indicated that good control could also be obtained with dieldrin applied by helicopter in the low-volume oil spray used in some areas for disease control.

The control practices reviewed above were chiefly on Gros Michel bananas. The variety grown by the Standard Fruit Company in the Aguan Valley of Honduras is the Giant Cavendish, which is a shorter plant and is resistant to Panama (*Fusarium* wilt) disease. Control of *Cercospora* leaf spot disease is accomplished by low-volume, helicopter applications of orchard spray oil every 2 to 3 weeks. The following control trial was established to evaluate the results of dieldrin mixed in this spray oil in terms of red rust control, phytotoxicity, and possible induced increases of other pests, when applied to the Giant Cavendish variety.

MATERIALS AND METHODS.—An area consisting of approximately 100 acres was selected from the most heavily infested area of a farm in the Aguan Valley. One-half of this area was sprayed with 0.8 gallon per acre of a mixture consisting of 1 quart dieldrin emulsifiable concentrate (1.5 pounds technical dieldrin per gallon) and the balance spray oil applied April 12 and again on April 26, 1960. The other half received only spray oil. Five 4-acre areas were selected inside the treated acreage and five similar adjacent areas in the untreated acreage for pre- and post-treatment counts of thrips and fruit damage. For these counts 20 stems, 1 to 4 weeks from harvest, were selected at random in each of the 10 sample areas. On each of 10 stems selected from the 20-stem sample the numbers of adult and immature thrips were counted on five randomly selected fingers. Also each stem was inspected for fruit-scarring caterpillar damage (Arctiidae—*Ecpantheria* sp., Tortricidae—*Platynota* sp.). Counts were made before the first application, between the first and second applications, and at approximately 2-week intervals thereafter for the first 4 months, after which counts were made monthly. Seven months after treatment it became necessary to spray the entire farm with dieldrin for red rust control and the experiment was terminated.

In addition to the control trial, counts of red rust thrips and red rust incidence were also made in various representative farm areas to relate thrips numbers with red rust incidence over a broad range of infestation levels and population trends. For this purpose 50 preharvest (1 to 4 weeks before harvest) stems were inspected in each of 17 20-acre areas selected in six plantations comprising a total of about 3,500 acres. Three sets of counts were made, 4 weeks apart.

RESULTS.—The results of 12 sets of counts in the 7-month period following the first dieldrin application in the control trial are summarized in figure 2. These indicate that two dieldrin applications practically eliminated the red rust thrips population. In spite of the proximity of a heavy infestation, the population in the treated area showed no signs of resurgence by the end of the experiment. The fluctuations in thrips population were reflected in the injury incidence. The fact that the injury represents the accumulative effect of recent and present populations, however, modified the fluctuations so that they were less severe and somewhat delayed. The delay in symptom expression was particularly evident in cases of drastic

population changes, such as the insecticidal reduction of thrips which occurred in the first 3 weeks after the first application but which was reflected only partially until the sudden drop in red rust 9 weeks after the first application. Such a marked delay, however, was not seen in normally fluctuating populations.

The counts of fruit-scarring caterpillar injury indicated no consistent differences between treatments. Repeated observations in the treated and untreated areas revealed no increase in any other banana pest as a result of the dieldrin applications during the course of this experiment. The fact that the quantity of active ingredient per acre is fairly low and the low volume oil application results in little of it penetrating the leaf canopy to the weeds below, very likely lessens the chances that this method of control will produce the adverse effects associated with the granule applications described above. No phytotoxicity to fruit or foliage could be detected.

In the series of inspections made in the 17 farm areas, the red rust incidence for the last set of counts was compared with the thrips counts in the same set, an average of the same and the past set, and an average of all three sets. The highest correlation ($r = 0.94$) was obtained by relating red rust incidence to thrips counts made at the same time. Since damage is accumulative over the latter portion of fruit development, one would, no doubt, expect cases in which damage at a given time more clearly reflects the level of a recent rather than the present population. Under the conditions of these counts, however, in which the average of a large sample of normally fluctuating populations of various levels was used, it was indicated that concurrent thrips and damage counts were well enough correlated to permit their use in estimating the amount of damage normally associated with various thrips populations. These relationships are plotted in figure 3. The use of the calculated line will enable growers to estimate the numbers of thrips associated with different levels of injury. Thus a treatment level can be established on the basis of thrips counts rather than injury severity, which is preferred on the basis of observations noted above—that a drastic decline in thrips populations is not fully reflected for some time in red rust incidence.

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Phytotoxicity of Wetting Agents on Lychee[1]

S. NAKATA and Y. TANADA,[2] *Departments of Plant Physiology and Entomology, University of Hawaii, Honolulu*

ABSTRACT

The lychee leaves are susceptible to both ionic and nonionic surfactants. The injury due to the wetting agents ranged from complete necrosis of leaves to chlorosis and mottling and premature leaf fall. Wheat flour suspension was found to be nontoxic and adequate as a surfactant, but its use at a high concentration increased the turbidity of the spray suspension. Iso-octyl phenoxy polyethoxy ethanol (Triton X-100) was the least injurious of the commercially available surfactants tested.

The lychee, *Litchi chinensis* Sonnerat, was first brought to Hawaii in 1873 from China (Neal 1948) and because of its delectable fruit, it is becoming a commercially important horticultural plant in Hawaii and in the continental United States, especially in Florida. Approximately 100 acres of lychee plants are established in Hawaii (Yee 1957) and more than 15,000 trees have been planted in Florida (Dekle 1954a). The recent approval of fresh lychee fruit shipment to the continental United States (Anon. 1959) and the results obtained by Akamine (1960) on the preparation of lychee fruits for export should stimulate further lychee production in Hawaii.

Lychee trees are attacked by a number of insect pests (Higgins 1917, Dekle 1954a, 1954b, Butcher 1955, Nishida & Holdaway 1955). One of the serious pests of lychee in Hawaii is the erinose mite, *Aceria litchii* (Keifer) = (*Eriophyes chinensis* Trotter, *E. litchii* Keifer), which may cause severe crinkle and deformation of the leaves. It is controlled easily, however, with the use of sulfur either as dust or as an aqueous suspension.

Occasionally the Chinese rose beetle, *Adoretus sinicus* Burmeister, may cause severe feeding damage to the foliage. This scarabaeid beetle is highly susceptible to DDT which has been used to control this beetle. When DDT is used on lychee, an infestation of scale insects may develop, one of which is the red wax scale, *Ceroplastes rubens* Maskell. In order to control scale insects, oil emulsions have been used on lychee trees, but they have caused severe injury to the trees, whose leaves gradually developed slight to intense chlorosis and fall off prematurely within 1 to 2 weeks. This indicates that the lychee foliage is highly sensitive to oil compounds.

Inasmuch as lychee leaves are smooth and waxy and difficult to wet, a wetting agent is necessary in insecticidal sprays in order to ensure a good coverage of the leaves with the spray. Early attempts with wetting agents containing a soap base (e.g., fish oil soap) had resulted in injury to the leaves. The present paper describes a series of tests conducted during the period 1956 to 1960 to evaluate the susceptibility of the lychee trees to various

[1] Published with the approval of the Director of the Hawaii Agricultural Experiment Station as Technical Paper No. 529.

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[2] Present address, Department of Insect Pathology, University of California, Berkeley.

proprietary wetting agents that are presently available commercially.

MATERIALS AND METHODS.—The Brewster variety of lychee was used in most of the tests, but occasionally other varieties, such as the Kwai Mi, Hak Ip and seedling lychees were used. The wetting agents were sprayed on branches, preferably those with both young and mature leaves and care was taken to thoroughly wet the leaves.

In the preliminary tests, the wetting agents were sprayed on the lychee foliage with hand-operated 3-gallon knapsack sprayers. The concentrations that were selected for application were ascertained first by dipping small branches with leaves into various concentrations of the wetting agents. The concentration that gave a thorough wetting of the entire upper and lower leaf surfaces was selected as the highest concentration to be used in the tests. Both young and mature leaves were used in this preliminary dip test. After the highest concentration had been determined, several lower concentrations of wetting agents were tested in like manner. These lower concentrations usually wetted the leaves to varying degree. Each concentration was applied to three different branches that were observed at weekly intervals for signs of phytotoxicity, i.e., mottling, leaf tip "burn" or necrosis.

In the initial test, methyl cellulose (Methocel), modified phthalic glycerol alkyd resin in ethylene dichloride—77% (Triton B-1956), iso-octyl phenoxy polyethoxy ethanol (Triton X-100), alkylphenolethylene oxide (Oronite NI–W), triethanolamine salt of dodecyl benzene sulfonate (CLE), sodium salt of dodecyl benzene sulfonate, polyoxyethylene ester of mixed fatty acids and resin acids (ER 10), sodium lauryl sulfate, and alkyl benzene sulfonate (Ultrawet), were used at dilutions given in table 1. In other series of preliminary tests, sulfonated aliphatic polyester (Nekal WS 25), sodium-n-methyl-n-olely laurate (Igepon T77 and Igepon T51) and alkylarylpolyoxyethylene, isopropanol, free fatty acids (Colloidal X-77) were sprayed on the lychee trees.

In the subsequent tests compounds that were phytotoxic were eliminated and the promising ones were sprayed on lychee trees with a power sprayer at a pressure of about 150 to 250 pounds per square inch. Five separate tests with three trees per treatment were conducted in these trials, and wettable sulfur (5 pounds per 100 gallons) was also incorporated in the spray program.

RESULTS.—In severe cases of phytotoxicity, the very young leaves which have not yet attained their green color may be "burned" severely and may turn completely black and drop from the trees within a few days. The mature leaves that have been sprayed generally do not show any necrotic injury but turn chlorotic and eventually fall off the trees prematurely. In moderate phytotoxic cases, the young leaves do not show necrotic damage, but at times a few short-holes may develop. As the leaves mature they begin to show some chlorosis and mottling.

In mild cases of phytotoxicity, the expanding leaves that have not reached full maturity and those that have recently matured but are not fully hardened develop mottling within 1 to 2 weeks. The mottling increases with time but the leaves do not drop off. However, there are indications that such damaged leaves may fall much earlier from the plant than undamaged healthy leaves.

Another symptom of injury is the tip "burn" on a leaf.

Table 1.—Results of preliminary tests on the phytotoxicity of wetting agents to the leaves of the lychee plant.

WETTING AGENT	MANUFACTURER	CONCENTRATION	DEGREE OF INJURY		
			None	Slight	Severe
		Series 1			
Methocel[a]	Dow Chemical	1:250		×	
		1:500		×	
		1:1,000		×	
Starch[a]		1:100	×		
		1:200	×		
		1:400	×		
Triton[b] B-1956	Rohm & Haas	1:250		×	
		1:500		×	
		1:1,000	×		
Triton[b] X-100	Rohm & Haas	1:2,000	×		
		1:4,000	×		
		1:8,000	×		
Oronite[b] NI-W	Oronite	1:500		×	
		1:1,000	×(?)		
CLE[b]	Pacific Chemical & Fertilizer Co.	1:1,000		×	
		1:2,000	×		
		1:4,000	×(?)		
ER-10[b]	Pacific Chemical & Fertilizer Co.	1:500		×	
		1:1,000		×	
Sodium lauryl sulfate[b]		1:500			×
Ultrawet[b]	Atlantic	1:300		×	
		1:400			
		Series 2			
Nekal WS 25[b]	Antara	1:1,000		×	
		1:2,000		×	
Igepon T 51[b]	Antara	1:500		×	
		1:1,000		×	
Igepon T 77[b]	Antara	1:500		×	
		1:1,000		×	
Colloidal X-77[b]	Colloidal Products Corp.	1:500		×	
		1:1,000		×	
		1:2,000		×	

[a] Concentration prepared as weight (g.) to volume (ml.).
[b] Concentration prepared as volume to volume (ml.).

This injury is characterized by a chlorotic spot at the tip of the leaf and appears as if a drop of the wetting agent had dried on the tip. The old mature leaves and very young leaves (leaves less than 1 inch in length) are the least affected.

Figure 1 illustrates the typical mottling and necrosis caused by some of the wetting agents. The degree of mottling and necrosis that developed was dependent on the type of wetting agents and the concentrations that were used.

Preliminary tests indicated that wheat flour and Triton X-100 were apparently nontoxic at the concentrations tested (table 1). Triton X-100 appeared to be the better wetting agent to use because it gave adequate wetting of leaves at very high dilutions. Wheat flour gave good wetting but required a large quantity of solid material that greatly increased the turbidity of the spray, especially when the insecticide was used as a wettable powder. The other wetting agents, CLE, Triton B-1956, and Oronite NI–W, were not injurious at low concentrations but at the dilutions required for adequate wetting they were toxic to the leaves.

The wetting agent, Triton X-100, was found to be slightly phytotoxic to both Brewster and seedling lychee varieties when applied with a power sprayer. When it was applied at the rates of 1:4,000 and 1:6,000, slight injury

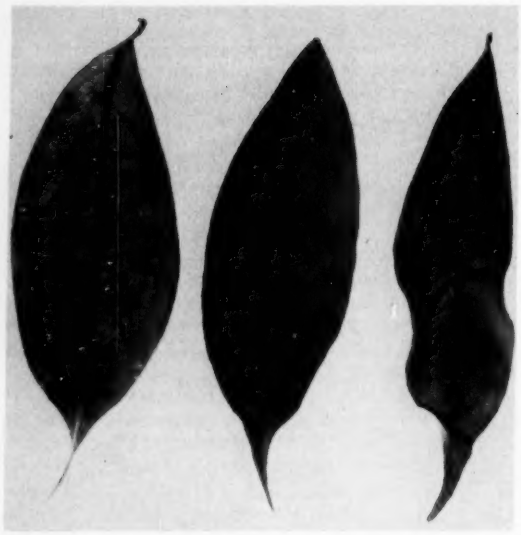

FIG. 1.—Effect of wetting agents on litchi leaves. Left, leaf mottled by Triton B-1956 (1:1,000); center, untreated normal leaf; right, leaf with necrosis and crinkle caused by Nekal WS 25 (1:1,000).

appeared in the form of slight mottling of leaves 2 weeks after the spray application regardless of whether sulfur was added to the spray. The injury on leaves that were sprayed with Triton B-1956 and sulfur was evident a week after spraying. Wheat flour at the rate of 1:250 (g. to ml.) dilution appeared to be the only one nontoxic to the leaves.

DISCUSSION.—It is common knowledge that many plants are susceptible to injury when sprayed with insecticidal oils and emulsions, but it is unusual to observe a plant, as in the case of lychee, that is highly susceptible to even proprietary surfactants. This has created a problem in the control of insect pests on lychee with the use of insecticidal sprays, because a wetting agent has to be incorporated in the spray in order to adequately wet and cover the smooth waxy leaves. Iso-octyl phenoxy polyethoxy ethanol (Triton X-100) was found to be the least injurious to the lychee of all the proprietary surfactants tested. The only material that showed no phytotoxic reaction on lychee was wheat flour. However, the disadvantage of using wheat flour was the high concentration (1 g. to 250 ml. of spray) required to obtain adequate wetting of the leaves. This high concentration of solids greatly increased the turbidity of the spray, especially when the insecticides were used in the form of wettable

Table 2. Results of the application of wetting agents to seedling lychee plants in November 1960.[a]

MATERIAL USED	CONCENTRATION OF WETTING AGENT	DEGREE OF WETTING	DEGREE OF INJURY[b]
Sulphur only	5 lbs./100 gal.	poor	none
Triton X-100	1:4,000	good	slight mottling on young expanding leaves only
Triton X-100[c]	1:4,000	good	slight mottling on young expanding leaves only
Triton X-100	1:6,000	good	slight mottling on young expanding leaves only
Triton X-100[c]	1:6,000	good	slight mottling on young expanding leaves only
Wheat flour[c]	1:250	good	none
Triton B-1956	1:4,000	fair	severe mottling on expanding leaves, but none on very young leaves

[a] The wetting agents were applied with a power sprayer at a pressure of 150 to 250 pounds per square inch.
[b] Condition of leaves at the time of spray application.
[c] Five pounds of sulphur/100 gal. added to the wetting agent.

powder. Although Triton X-100 caused only very slight injury and could be used on lychee, there is a definite need for further search for more satisfactory surfactant agents.

The present study also indicated that insecticidal sprays of materials used in the form of emulsions that contained oil solvents and emulsifiable agents might also cause injury to lychee foliage.

No attempt was made to determine the mechanisms by which oils or oil-like materials and ionic and nonionic wetting agents cause injury to the lychee foliage. Such studies may not only assist in the selection of suitable wetting agents for the lychee plant, but may also provide valuable information on the absorption of material by the plant through the leaves.

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Resistance to the Potato Leafhopper in Lima Bean Lines, Interspecific *Phaseolus* Crosses, *Phaseolus* spp., the Cowpea, and the Bonavist Bean[1]

DAN WOLFENBARGER[2] and J. P. SLEESMAN,[3]
Ohio Agricultural Experiment Station, Wooster

ABSTRACT

Nineteen lima bean lines were resistant to hopperburn, although the varieties differed in resistance to nymphal infestation. All plants or interspecific crosses of resistant and susceptible species were susceptible to both hopperburn and nymphal infestations. Unidentified *Phaseolus* species differed in their reaction to both nymphal infestations and hopperburn injury. The Bonavist bean was resistant to hopperburn but susceptible to nymphal infestation. Although cowpea lines exhibited tolerance they were susceptible to nymphal infestations and hopperburn injury.

Response to potato leafhopper (*Empoasca fabae* (Harr.)) attack varies greatly among and within the legume genera *Dolichos*, *Vigna*, and *Phaseolus*. The purpose of this investigation was to determine how much variation exists within and among the various genera under natural infestations in the field.

DeLong (1938) described hopperburn symptoms of common bean, *Phaseolus vulgaris* L. Poos & Wheeler (1943) stated that the potato leafhopper was reared from *Phaseolus lunatus* var. *macrocarpus* Benth.; mung bean, *Phaseolus aureus* Roxb.; rice bean, *Phaseolus calcaratus* Roxb.; Bonavist bean, *Dolichos lablab* L.; and cowpea, *Vigna sinensis* (Torner) Savi.

Meader (1960) stated that the Scarlet Runner bean, *Phaseolus coccineus* Willd., is susceptible to hopperburn injury. One worker (Anon. 1940) stated that plants of small-seeded type lima bean were damaged by the leafhopper *Empoasca fabalis* DeLong whereas plants of the large-seeded sorts were not damaged. McCrea bush lima is a selection from a cross between a large and small-seeded variety. The foliage is similar to that of the large-seeded variety and leafhopper injury was intermediate between that of the large and small-seeded varieties (Anon. 1940). McFarlane & Rieman (1943) stated that Henderson bush lima showed outstanding resistance to

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[2] Present address: Texas Agricultural Experiment Station, Substation No. 15, Weslaco, Texas.
[3] Research Assistant and Professor, respectively.
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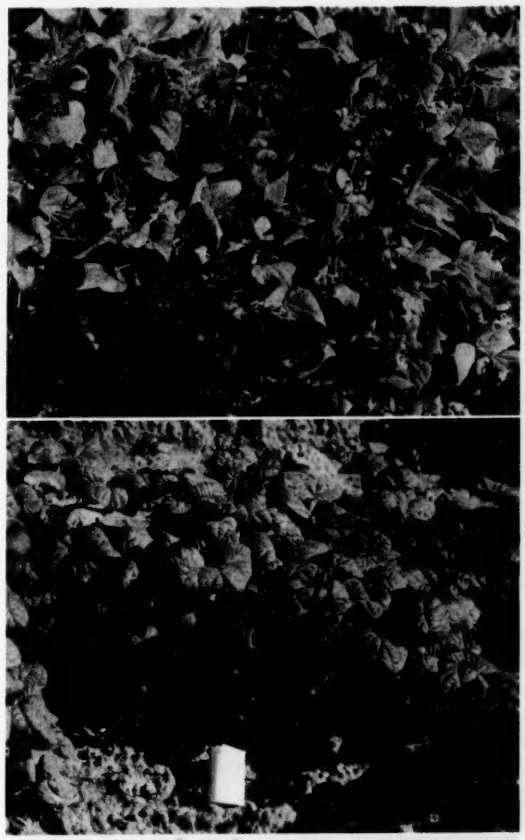

FIG. 1.—Classes of relative hopperburn damage by the potato leafhopper (Top) Class 0 showing no injury (Idaho Refugee); (Middle) Class 2 showing a curling or cupping of leaves (*P. vulgaris* PI 181893); (Bottom) Class 4 showing symptoms in Class 2 plus a yellowing of the leaves starting at the outer margins (*Glycine max* T145).

Table 1.—Reaction of *Phaseolus* spp., *D. lablab, V. sinensis* varieties, accessions, or strains, and interspecific *Phaseolus* crosses to the potato leafhopper, Marietta and Wooster, Ohio. 1959–60.

Accession, Variety, Species, or Strain	Nymphs per Trifoliate Leaf[a]							Class Hopperburn[b]				
	Marietta			Wooster				Marietta	Wooster			
	1959	1960		1959		1960		1959	1959	1960		
	July 6–8	July 11–12	July 27	July 13–16	Aug. 10	Aug. 1–2	Aug. 8–9	July 23	Aug. 3	Aug. 16	Mean Nymphs	Mean Class Hopperburn[b]
P. aureus PI 212907	0	0	0	0	0	0	0	0	0	0	0	0
P. aureus PI 217959	0	0	0	0	0	0	0	0	0	0	0	0
P. aureus PI 219699	0	0	0	0	0	0	0	0	0	0	0	0
P. aureus PI 223711	0	0	0	0	0	0	0	0	0	0	0	0
P. lunatus PI 194314	0.1	0	0	0	0	0	0	0	0	0	0	0
P. lunatus PI 195340	0.1	0	0	0	0	0	0	0	0	0	0	0
P. lunatus PI 209051	0	0	0	0	0.3	0	0	0	0	0	0	0
P. lunatus Dixie Butterpea		0			0					0	0	0
P. lunatus PI 180324	0	0.7	0	0	0.3	0	0	0	0	0	0	0
P. lunatus PI 195341	0	0	0	0	0.7	0	0	0	0	0	0	0
P. mungo PI 101581	0.3	0	0.3	0	0			0	0		0.1	0
P. radiatus 57703		0	0			0.3	0			0	0.1	0
P. radiatus 57704	0.4			0	0	0	0	0	0	0	0.1	0
P. lunatus King of the Garden		0.7	0			0	0			0	0.2	0
P. lunatus PI 198092	0.3	0.3	0.3	0.3	0.7	0	0	0	0		0.3	0
P. lunatus Jackson's Wonder Bush	0.7			0	0	0.7	0.3	0	0	0	0.3	0
P. lunatus 4913		1.0	0.3			0	0.3			0	0.4	0
P. lunatus Fordhook C2	0	0	0	0	0	3.0	0	0	0	0	0.4	0
P. lunatus Clark's Bush		0.7	0			0.7	0			0	0.4	0
P. lunatus Piloy	1.7	0			0	0	0			0	0.4	0
P. atropurpureus		0	0.7								0.4	—
Phaseolus sp. PI 213017		1.0	0.7			0	0			0	0.4	0
P. lunatus Thaxter		1.0	0			0	1.0			0	0.5	0
P. mungo PI 174907		0	2.0			0	0			0	0.5	0
P. lunatus PI 195344	0.9	0	0	0.5	2.7	0	0	0	0	0	0.6	0
P. lunatus Henderson Bush	2.3			0.5	0	0	0	0	0	0	0.6	0
P. lunatus Burpee's Improved Bush	0.1	1.0	0	0	0	3.3	0	0	0	0	0.6	0
P. lunatus Fordhook 242	0	0	0	0	0	4.3	0	0	0	0	0.6	0
P. calcaratus	2.1	2.7	0	0	0	0	0	1.0	0	0	0.7	0.3
P. aureus PI 207504	0.4	0.7	2.9	0	1.0	0.3	0.3	0	0	0	0.8	0
P. lunatus Wood's Prolific		2.0	0			0.3	1.0			0	0.8	0
V. sinensis 18-17-2110	5.9	0.3	0	0	0			4.0	0		0.9	2.0
P. lunatus Thorogreen	2.6	2.3	2.7	0.3	1.0	0	0.3	0	0	0	1.3	0
P. aureus PI 164889	1.3	7.7		0.5	0	0	0	0	0		1.6	0
P. aureus 57705	3.9					0	1.0	0		0	1.6	0
P. lunatus Florida Butter Speckled		2.3	0.3			0	9.3			0	3.0	0
P. acutifolius PI 200920	10.3	5.3		2.3	2.3	0.3	1.7		2.5	2.0	3.7	2.3
P. lunatus Sieva		5.7				0.7	6.7			0	4.4	0
D. lablab Bonavist Bean		1.0	7.7			0	0			0	4.4	0
V. sinensis 644210	7.1	11.7		1.5	1.7	1.3	5.0	4.0		0	4.7	2.0
V. sinensis 73-05110	9.7	10.0		0	1.0			4.0	0		5.2	2.0
V. sinensis Brabham		1.7	17.3			0.3	3.0			0	5.6	0
P. vulgaris×*P. mungo* No. 12 throwback		6.3	5.7								6.0	—
P. acutifolius (T_4×T_{1360})15.6 Tepray F_4 gen.	15.6			1.3	1.7			4.0	1.0		6.2	2.5
P. vulgaris×*P. mungo* No. 12 segregating		12.7	5.7			7.3	4.0			2.0	7.4	2.0
Phaseolus sp. PI 224746	14.7	10.3		3.0	2.7	1.7	3.7	4.0	0.5	0	9.0	1.5
P. coccineus×*P. lunatus*	26.1							4.0			26.1	4.0

[a] Sampled three trifoliate leaves per plot.
[b] Based on 0 least, 4 most.

the potato leafhopper, and Gui (1945) reported that lima bean varieties were less densely populated by leafhoppers than were snap beans.

Material and Methods.—At Marietta and Wooster, Ohio, in 1959 and 1960, 20 to 40 seeds of the various lots were planted in single 5-foot rows with 3 feet separating the plots in and between the rows.

The method used in estimating leafhopper populations was to count the nymphs on trifoliate leaf samples taken from each plot. Lines with a mean of 0.4 nymph or less per trifoliate leaf were considered resistant. Lines exhibiting 0.5 to 1.5 nymphs per trifoliate leaf were classed as

having a low order of resistance. All lines with more than 1.5 nymphs per trifoliate leaf were considered susceptible.

In 1959–60, hopperburn damage symptoms were recorded in five classes as follows:

Class 0 = No injury.
Class 1 = Slight injury (little curling or rolling of leaves).
Class 2 = Medium injury (curling or rolling of leaves).
Class 3 = Heavy injury (symptoms same as Class 2, plus stunting and slight yellowing of leaf edges).
Class 4 = Very heavy injury (symptoms same as Class 3, plus severe yellowing of the leaves).

In the relative hopperburn damage scale, lines that were rated 0 are resistant, lines that were rated 4 are considered highly susceptible.

Figure 1 illustrates Classes 0, 2, and 4 of the described visual ratings.

RESULTS AND DISCUSSION.—The mean nymphal populations for *Dolichos*, *Vigna*, *Phaseolus* spp., and interspecific *Phaseolus* crosses are ranked from low to high in table 1.

In table 1 Sieva bean is described as the lima bean most susceptible to nymphal infestations. Sieva, Florida Butter Speckled, and Thorogreeen are small-seeded limas and they were susceptible to *E. fabae* infestations. However, Dixie Butterpea, Thaxter, Jackson's Wonder Bush, and Early Thorogreen also are small-seeded and yet they were resistant to nymphal infestation. One worker (Anon. 1940) stated that large-seeded limas were resistant to injury. None of the lima bean lines showed hopperburn injury; therefore, seed size would not seem to be a factor contributing to resistance in limas to *E. fabae*. Several Plant Introductions (PI) and commercial lines were resistant to nymphal infestations. *P. lunatus* L. PI 194314, 195340, 209051, 180324, 195341, and Dixie Butterpea were almost immune to attack by the potato leafhopper. Common cowpea strains were susceptible to hopperburn injury only when nymphal populations were high, indicating a tolerance to the leafhopper. This tolerance was shown by three cowpea strains (73-05110, 18-17-2110, and 644210) which had populations that ranged from 5.9 to 9.7 nymphs per trifoliate leaf at Marietta in 1959 and a corresponding hopperburn rating of 4. When these same strains had populations of 0 to 5 nymphs per trifoliate leaf, they received hopperburn ratings of 0 at Wooster in 1959–60.

Scarlet Runner bean, *Phaseolus coccineus*, lines (Meader 1960) and a susceptible *P. coccineus* × resistant *P. lunatus* interspecific cross from S. Honma were highly susceptible to hopperburn and nymphal infestations. This was true also for the resistant mungo bean cross (*Phaseolus mungo* L. × susceptible Asgrow Stringless Green Pod *P. vulgaris* throwback) and a segregating interspecific cross from A. B. Strand. This indicates that resistance to leafhopper injury and infestation is recessive at least for the lines used in these evaluations.

Table 2.—Mean nymphs per trifoliate leaf and mean visual ratings of *Phaseolus* spp., interspecific *Phaseolus* crosses, and related genera, Marietta and Wooster, Ohio. 1959–60.

SPECIES	NO. VARIETIES EVALUATED	NO. EVALUATIONS	MEAN NUMBER NYMPHS	NO. EVALUATIONS	MEAN HOPPERBURN INJURY[a]
P. radiatus	2	9	0.1	4	0
P. mungo	2	10	0.3	2	0
P. atropurpureus	1	2	0.4	—	—
P. aureus	13	86	0.5	37	0
P. lunatus	33	187	0.7	79	0
P. calcaratus	1	7	0.7	2	0
D. lablab	1	4	2.2	1	0
V. sinensis	4	19	4.1	7	1.7
P. acutifolius	2	9	4.5	4	2.4
P. vulgaris × *P. mungo*	2	6	7.0	1	2.0
P. coccineus × *P. lunatus*		1	26.1	1	4.0

[a] Based on 0 least, 4 most.

All *Phaseolus radiatus* L. bean lines were resistant to nymphal infestation and hopperburn. Several mung bean, *Phaseolus aureus*, Plant Introductions were immune to nymphal infestation whereas others were susceptible. All domestic mung beans were resistant to nymphal infestation and hopperburn injury. *Phaseolus* sp. PI 224476 was susceptible whereas *Phaseolus* sp. PI 213017 was resistant to nymphal infestation and hopperburn injury. *Phaseolus atropurpureus* DC. was resistant to nymphal infestation. The rice bean (*Phaseolus calcaratus*) was low in resistance to hopperburn and nymphal infestation. Tepray beans (*Phaseolus acutifolius* A. Gray) were susceptible to nymphal imfestation and hopperburn.

In table 2 the data in table 1 are summarized and the various species are ranked according to mean nymphal counts and hopperburn injury. These data indicate that several *Phaseolus* species and the Bonavist bean are free from hopperburn. Lines of mung bean and lima bean lines vary in nymphal infestation, but the mean nymphal infestation indicates that they are resistant. The Bonavist bean is susceptible to nymphal infestation.

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Sequential Plans for Sampling Aphids on Sugar Beets in Kern County, California[1]

EDWARD S. SYLVESTER[2] and EDWIN L. COX[3]

ABSTRACT

An efficient sampling plan, to aid in the timing of treatments, is essential to the development of a practical insecticidal program for possible use in the control of *Myzus persicae* (Sulzer), the major vector of the beet yellows viruses. Sequential sampling, in which decisions are made upon the accumulated results of each unit sampled, in many cases can save both time and money. Although the tendency of aphids to colonize individual plants leads to what is known as over-dispersed, or contagious, distributions, critical population levels (assumed to be approximately one aphid per plant) are reached before 100% of the plants are infested. This fact permits the use of sequential sampling plans based upon either the mean number of aphids per plant or the number of plants found to be infested. In situations of very light or very heavy populations the use of a sequential sampling plan should allow rapid decisions to be made as to whether critical population levels have been reached.

Traditionally, sugar beets have been grown in Kern County, California, without costly applications of insecticides. However, the losses in both tonnage and sugar content due to infection with yellows viruses may justify the expenditure of money for aphid control, if it can be shown that virus spread can thus be reduced and the number of insecticide applications needed is economically feasible. That virus spread can be reduced and tonnage and/or per cent sugar increased by vector control is an accepted fact among research workers in England (Hull & Gates 1953), Europe (Dame & Goossen 1954), and the United States (Bennett et al. 1954, Sylvester et al. 1959). That such procedures are profitable under all conditions is uncertain (Hull & Gates 1953).

The effectiveness of virus conrol programs depends upon many factors (Broadbent 1948), and in the case of beet yellows, at least two of these are of particular importance, viz., (1) low incidence of virus in immigrating alatae, and (2) the proper timing of insecticidal applications to prevent within the field population increase. In Kern County the recommendation of a beet-free period (Bennett & Costa 1954, Sylvester et al. 1959) was aimed at reducing the number of virus-carrying spring alatae. This is still a sound recommendation in spite of the fact that radish yellows, a virus which exists independent of sugar beets (Duffus 1960), may be present.

In the present paper, two sequential sampling plans to aid in the timing of insecticide applications will be examined.

STATISTICAL CONSIDERATIONS.—The data were from population studies made during the springs of 1958 and 1959 on the development of aphids, mainly the green peach aphid, *Myzus persicae* (Sulzer), on sugar beets in Kern County, California. The experimental areas of 1958 have been described previously (Sylvester et al. 1959), while that of the 1959 study consisted of 4 treatments, 4 replications. Each plot included eight rows of commercially grown sugar beets. The plots were 200 feet in length and began 50 feet from the edge of the field. Weekly sampling was begun on March 3, and continued at weekly intervals for a total of 9 weeks. The sampling procedure was as previously reported (Sylvester et al. 1959) viz., 100 plants per plot during the prethinning period and 40 plants per plot during the postthinning period. At no time was the number of plants sampled per plot reduced to 10 as was the case in 1958 when populations were considerably higher.

During initial phases of infestation when alatae are locating on the plants, the distribution of the insects might be considered to follow a Poisson distribution (mean = variance) since the probability of finding an aphid is relatively small, and the distribution of the alighting alatae might be assumed to be independent and at random. However, since the alatae begin to reproduce almost immediately, the Poisson distribution model is rapidly replaced by a compound Poisson, contagious, or overdispersed distribution (table 1). The best evidence of this phenomenon is the nonlinear yet dependent relationship between the mean and the variance (fig. 1).

Several mathematical models are available to handle the problem of contagion or overdispersion (Bliss 1958), but recent emphasis has been placed upon the usefulness of the unimodal negative binomial distribution in connection with entomological data (Fisher 1941, Anscombe 1949, Bliss & Fisher 1953, Morris 1955, Bliss 1958). This is especially true in connection with certain forest entomological problems and the development of sequential sampling methods (Morris 1954, 1955).

In spite of the fact that the negative binomial distribution does not have the causal implicative content that frequently is assumed in connection with either the Poisson or the binomial distributions (Bliss 1958), the usefulness of it in estimation of variance, data transformation, the development of sequential sampling plans (Oakland 1950), and the advantage of the relative simplicity of its calculations, all have been adequately evidenced. This is especially true if the insects concerned have a period of population stability in any one sampled space so that the samples collected during such times are of good predictive value and space is one of the more important variables.

The negative binomial distribution, or in general all distributions specifying the probabilities of 0, 1, 2, · · · etc. favorable or unfavorable occurrences may be condensed to the dichotomy: probability of less than or equal to and probability of greater than some for example zero,

[1] Accepted for publication March 27, 1961.

[2] Associate Entomologist, University of California, Berkeley.

[3] Biometrician, Biometrical Services—Agricultural Research Service, Plant Industry Station, Beltsville, Maryland.

Table 1.—Means (aphids per plant) and variances calculated from data collected during the spring of 1958 and of 1959 on sugar beets in Kern County, California, illustrating the rapid departure from a Poisson distribution model (mean = variance).

	Location[b]							
	Rockwell		Camp and Lackenmaier		Henson Bros.		Kern Land	
Sample[a]	Mean	Variance	Mean	Variance	Mean	Variance	Mean	Variance
1	0.0025	0.0025	0.00	0.00	0.005	0.005	0.00	0.00
2	0.025	0.059	0.028	0.047	0.0025	0.0025	0.001	0.001
3	0.09	0.16	0.135	0.295	0.138	0.157	0.013	0.035
4	1.18	2.82	0.150	0.358	0.181	0.602	0.035	0.041

[a] Samples for Rockwell, Camp and Lackenmaier, and Henson Bros., were taken on February 11, 18, 25, and March 4, 1958; those for Kern Land were taken on March 3, 10, 17, and 24, 1959.

[b] The locations are designated by the grower. Rockwell was near Wasco; Camp and Lackenmaier and Kern Land, near Shafter; and Henson Bros., in the Maricopa Flats area southwest of Bakersfield.

number of occurrences. Hence, if a negative binomial distribution describes aphid infestation, the expected or mean number of aphids per plant can be related to the expected percentage of plants infested. In the present work (table 4) when 50% of the plants were infested the average number of aphids per plant approached 1, while as the per cent of infested plants neared 100, the mean number of aphids per plant had increased to approximately 18 aphids. Broadbent (1948) found on potatoes in England that when 10% of the sample units (1 unit being 3 leaves, one from each of the lower, middle, and upper portions of the plant) were infested, the populations were equal to or less than 20 aphids per plant. Shands & Simpson (1959), using a similar sampling technique, concluded that on potatoes in northeastern Maine when 39% of the sample units were infested, the average aphids per plant was approximately 2.5, whereas when 100% of the plants were infested, the mean aphids per plant was 75 (range 13 to 200).

Anscombe (1948) used the negative binomial distribution in connection with the estimation of aphid populations in potato fields, and in addition provided a formula for the calculation of the standard error of the estimate. The generating function for the negative binomial distribution is often written $(q-p)^{-k}$ where $q=1+p$; the mean $m=kp$ and the variance $\sigma^2=kpq$. It has been suggested that the exponent k is related to the intrinsic power of a species to reproduce. However, it has been noted that the value of k tends to increase with the mean (Anscombe 1949, Morris 1954), and Bliss (1958) was of the opinion that a single k would not necessarily characterize a species.

With aphids, the multiplicity of generations, the brevity of the generation time, in addition to the problem of encountering more than one species on a given host plant, all tend to increase the importance of the space and time dimensions. The problem of estimating a valid k and one which could be considered to be common to the population even for short sampling periods obviously becomes both hazardous and complex. For example in the present work, using the data for 1958 (fig. 1) and that of 1959 (fig. 1), and the formula $k=(\Sigma\bar{x}^2)/(\Sigma s^2-\Sigma\bar{x})$, a value which would appear reasonably appropriate for the 1958 season is 0.55. A similar estimate for the 1959 season is 0.42. If three values of sample variance which were equal to or greater than four are ignored, this latter estimate approaches 0.55, an interesting correspondence.

However, when the data for 1959 are combined and considered as a single sample (table 2), the value of k falls to 0.099 because of the large zero class, a factor which is not of such importance in the above method of approximation. This lack of correspondence between estimates would indicate that a common k is not appropriate in this situation of weekly sampling.

Further evidence that a common k is not suitable can be emphasized from the comparison of the observed relationship between the mean number of insects per plant and the per cent of infested plants, and that percentage which would be expected with a given value of k.

By use of the large sample relationship

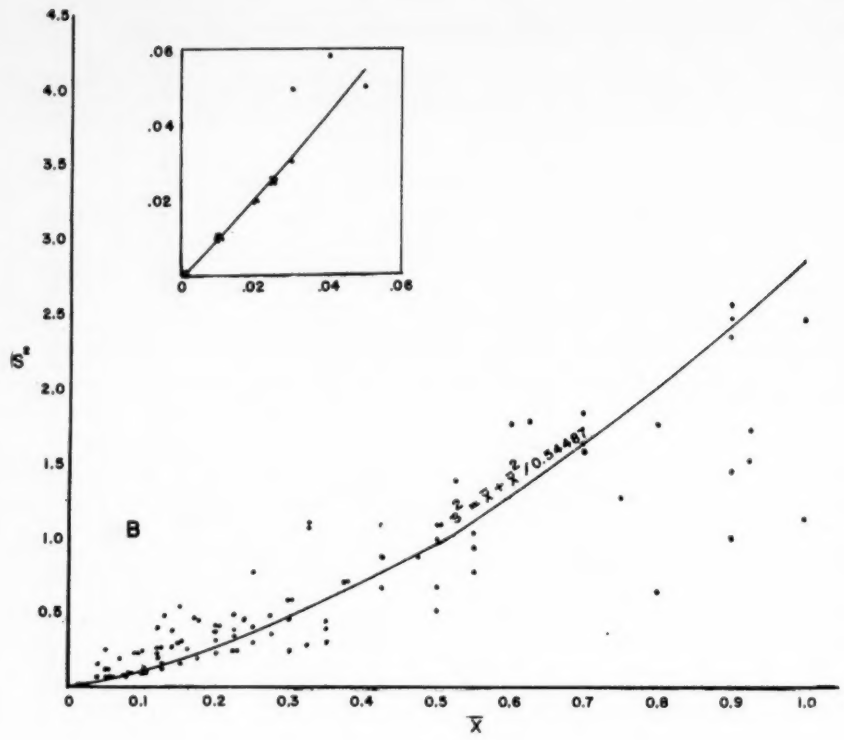
$$\log\left(1-\frac{I}{100}\right) = -k\log\left(1+\frac{\bar{x}}{k}\right)$$

where I is the per cent of infested units, $\bar{x}$ is the mean number of aphids per plant, and k is the negative binomial parameter, the expected values of I for selected values of $\bar{x}$ with $k=0.099$, 0.55 or 0.81 are given in table 3. The discrepancy between the observed per cent of in-

Table 2.—Fit of a negative binomial distribution to samples of aphids found on beets in Kern County, California, during the period March 3 through April 28, 1959.

Aphids/Plant x	Observed Frequency f	Accumulated Frequency A_x	Expected Frequency[a] k_1 (0.07652)	k_2 (0.09954)	k_3 (0.09938)
0	7866	774	7935	7866	7866.33
1	480	294	407.38	477.99	477.49
2	155	139	(no need to calculate)	160.43	160.33
3	84	55		68.54	68.53
4	29	26		32.42	32.44
5	9	17		16.23	16.25
6	4	13		8.42	8.44
7 (or more)	5 (13)	8		9.97	10.19
8	1	7			
9	3	4			
10	1	3			
11	0	3			
12	1	2			
13	0	2			
14	1	1			
15	1				
16+	0				
Sum	8,640		$1,378\chi^2=13$, d.f. $=5$ $p=<0.05$	10.48 $p=>0.05$	10.39 $p=>0.05$

[a] $k_1=(\bar{x}^2-s^2/N)/(s^2-\bar{x})$, k_1 was estimated by approximating the equation: $k=(\log N/f_0)/[\log(1+\bar{x})]$, where f_0 = observed frequency of the zero class; while k_3 was estimated by approximating the equation $z=\Sigma[(A_x/(k+x)] - N \ln(1+\bar{x}/k)$, where A_x = accumulated frequency in all units containing more than x organisms, x = class, and ln = natural log (common log times 2.3025851). For procedural details see Bliss & Fisher (1953).


B
$S^2 = \bar{X} + \bar{X}^2/0.54487$
S^2
$\bar{X}$

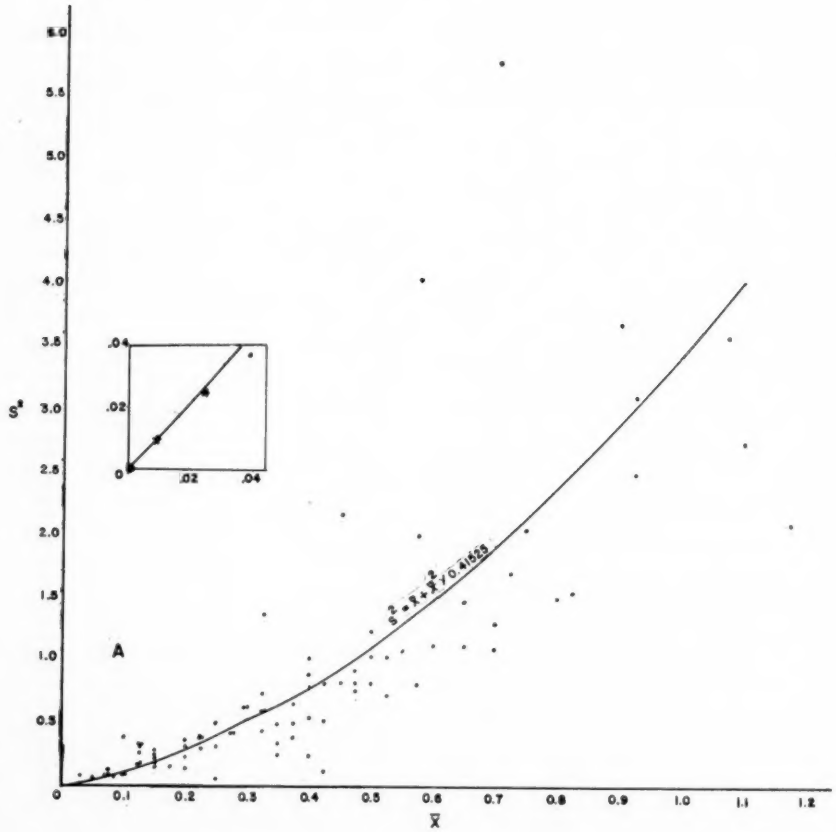

A
$S^2 = \bar{X} + \bar{X}^2/0.41629$
S^2
$\bar{X}$

Table 3.—Comparison of expected percentages of infested plants under the assumptions of $k=0.009$, 0.55 or 0.81 with the observed percentages found during a 9-week sampling study of aphids on sugar beets in the spring of 1959 in Kern County, California.

Mean Number of Aphids Per Plant	Per Cent Plants Infested			
	Observed	Expected with[a]		
		$k=0.099$	$k=0.55$	$k=0.81$
0.46	25.8	15.94	28.42	30.53
0.70	33.13	18.67	42.41	39.62
1.18	51.87	22.38	46.75	51.72

[a] The expected values were calculated from the large sample relation

$$\log\left(1-\frac{I}{100}\right)=-k\log\left(1+\frac{\bar{x}}{k}\right)$$

in which

I = per cent of infested plants
k = negative binomial parameter

and

$\bar{x}$ = mean number of aphids per plant.

fested units and that expected is clearly evident. Since both the mean number of aphids per plant and the per cent of plants infested were available for each week of the 9-week sampling period during 1959, an approximation of the value of k for each week was made using the above relationship. The results are given in table 4. From this it would seem unlikely that a common k could be applied, although there is some evidence that k tended to stabilize to a value between 0.7 and 1 over a range of infestation between 40% and 80%. Assuming that this range includes the critical period during which control measures should be initiated in order to intercept a developing vector population (either a mean of approximately 1 aphid per plant, or 50% infested units), and assuming that the mean value of k during this period (0.81) is appropriate and relatively constant, then a sequential sampling plan can be utilized in survey work.

Basically, sequential sampling as opposed to fixed sample size is a procedure whereby the sampler examines the units in sequence, with decisions being made on the information gained from each observation. If no acceptance or rejection decision is possible on the basis of the information from any given sample, the results are added to those obtained from the next and the process continued until a decision can be made.

Two approaches can be used in the present situation as to sequential sampling plans. One is based upon an assumption of a negative binomial distribution and involves counting all aphids found on each plant sampled, while the other is based upon the assumption of a binomial distribution and the plants examined are judged as either infested or noninfested, without counting the number of aphids found. Both will be presented, since each has its advantages. Using equivalent criteria, in the absence of an infestation, the minimum number of plants which must be examined before accepting this fact is the same in both plans, but from a field standpoint, the individual sampler may find it generally easier to count infested and noninfested plants than to count aphids. However in this case (the binomial), the average number of plants required to make a decision will be larger than in the plan based upon the negative binomial.

Table 4.—Successive changes in the value of k based upon weekly samples of aphids on sugar beets in Kern County, California, during the spring of 1959.

Per Cent Infested		Mean Aphids Per Plant	Estimate of k[a]
Range	Average		
0–5	1.23	0.054	0.005
6–10	8.25	0.13	0.1
11–15	13.75	0.25	0.15
16–20	17.08	0.27	0.25
21–25	22.5	0.42	0.15
26–30	25.8	0.46	0.3
31–35	33.13	0.7	0.4
36–40	38.33	0.75	0.6
41–45	42.5	0.73	1.1
46–50	48.75	1.18	0.7
51–55	55.0	1.18	1.05
56–60	58.97	1.74	0.7
61–65	64.06	2.23	0.75
66–70	70.00	3.33	0.7
76–80	80.0	5.46	0.75
86–90	90.0	7.48	1.15
91–95	90.63	6.56	1.3
96–100	98.33	18.78	1.6

[a] The estimates are approximations obtained by approaching the solution to the equation

$$\log\left(1-\frac{I}{100}\right)=-k\log\left(1+\frac{\bar{x}}{k}\right)$$

in which I = per cent infested plants and $\bar{x}$ = mean aphids per plant.

Use of the Negative Binomial Distribution.—In order to use any sequential sampling plan, three things are needed: (1) the frequency distribution of the items, (2) a realistic interval to separate the desired population levels, and (3) a decision as to the probability levels at which the sampler is willing to chance making an error in judgment.

The following assumptions were made to develop a sequential sampling procedure based upon the negative binomial distribution:

1. Populations averaging one aphid per plant were considered to be at a critical level as far as the initiation of insecticidal correctives in a vector control program. This is somewhat in excess of that used in Germany (30 to 50 aphids per 100 plants (Dame & Goossen 1954), but approaches that recommended in England for alates (Hull 1958)).

2. An average density equal to 0.9 or less aphids per plant was the "no treatment" limit.

3. An average density equal to 1.1 or more aphids per plant was the "treatment" limit.

4. The probability of failing to recommend a needed treatment was 0.10.

FIG. 1.—Relationship between the mean and the variance of small-plot (200×10 ft.) samples taken to estimate green peach aphid populations on sugar beets in Kern County, California. (A) Data from a single field during a 9-week period in spring of 1959, (B) from three locations during spring of 1958. Samples in which the mean exceeded 1.0 are not included.

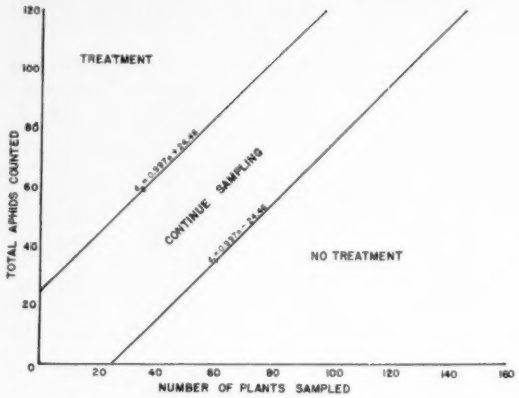


FIG. 2.—Acceptance and rejection lines for a sequential sampling plan based upon aphid counts on sugar beets assuming a negative binomial distribution of the population.

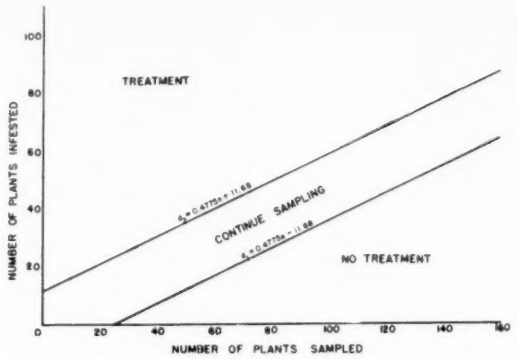


FIG. 3.—Acceptance and rejection lines for a sequential sampling plan based upon assuming a binomial distribution of infested and noninfested plants.

5. The probability of recommending an unnecessary treatment was 0.10.

6. The estimate of k equal to 0.81 would hold for the critical sampling period.

Using these assumptions it can be shown (fig. 2) that the minimum number of plants which would have to be counted without finding an insect would be approximately 25, with an average sample of something in excess of 200 plants being required when the population mean has an actual value of 0.9 aphids per plant.

USE OF THE BINOMIAL DISTRIBUTION.—Since critical population levels are reached before all plants are infested, a sequential plan based upon the binomial distribution of infested and noninfested plants might be advantageous in certain situations. For comparative purposes the criteria for decision were made equivalent to those used in the negative binomial, and were as follows:

1. If 45.44% or less of the plants are infested the decision would be "no treatment."

2. If 50.07% or more of the plants are infested the decision would be "treatment."

3. The probability of failing to recommend a needed treatment is 0.10.

4. The probability of recommending an unnecessary treatment is 0.10.

The decision lines resulting from these assumptions is illustrated in figure 3. Again if approximately 25 plants are sampled and no aphids found, the hypothesis of "no treatment" would be accepted. However, if the population was actually at the 45% level of infestation, an average of something in excess of 400 plants would have to be examined in order to reach a decision.

It is evident that in the early spring when the population of aphids on sugar beets in Kern County is very light, considerable time and effort could be saved in field survey work using a sequential sampling instead of one which required a fixed sample size. The same is true when the population became fairly heavy. Sampling would become most demanding as the critical level of the population is approached, but this is precisely when the demand for accuracy in judgment is at its highest. To be workable sampling should be done in those areas of the fields most likely to be infested. In Kern County, while many more data are needed, the aphid infestations were usually found near the edges of the fields. Limited observations indicate that the populations tend to grade in severity from west to east.

As with any sequential sampling plan the possibility exists that the sampler would continue to fall in the "continue sampling" zone, and in theory never be able to make a decision. However, some arbitrary maximum sample size (say 200 plants) could be set. If, after counting this number of plants, a decision still could not be made, the field would be sampled within the next day or two to determine its status. In actual practice in connection with sequential sampling plans, it has been found (Morris 1954) that tables made from tabulations from the lines are more easily used by workers than requiring them to plot points on a graph.

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Further Studies on the Feeding and Oviposition Preferences of the Boll Weevil (*Anthonomus grandis*)[1]

S. G. STEPHENS, *Genetics Department, North Carolina State College, Raleigh*
and
HONG SUK LEE, *Seoul National University, Suwon, Korea*[2]

ABSTRACT

Laboratory tests showed that boll weevils (*Anthonomus grandis* Boheman) did not discriminate between flower buds from red and other strains of cotton. In similar tests, when offered the "choice" of glandless and normal buds, they exhibited a preference for the normal condition. When a "choice" between glandless and normal was combined with a "choice" between hairy and nonhairy buds, preference was confined to the nonhairy types, with no discrimination between glandless and normal.

A field test is reported in which four strains of cotton (a standard Upland variety and three mutant strains, hairy, hairy-glandless, and hairy-glandless-red) were compared. All hairy strains received less damage than the standard Upland variety, but the weevils did not discriminate between the hairy and hairy-glandless strains. Data from the hairy-glandless-red strain were incomplete, but suggested that the inclusion of the red character in a hairy strain might increase resistance to boll weevil attack.

The resistance associated with hairiness could be separated into two categories:

1. Mechanical resistance, due to the fact that the bracteoles of hairy plants are "sealed" during the early stages of development, thus protecting temporarily the enclosed flower buds from damage.
2. Presumed antibiotic effects—larvae were found to develop less rapidly in hairy than in nonhairy buds.

Previous studies by Wannamaker (1957) and Wessling (1958a, 1958b) have shown that mutant strains of Upland cotton with exceptionally hairy buds and bracteoles suffer less damage from attack by boll weevil (*Anthonomus grandis* Boheman) than do normally hairy varieties. Preliminary tests in the laboratory (Stephens 1959) suggested that oviposition was influenced also by the presence or absence of resin glands. Weevils offered the "choice" of buds from two mutant strains, "glabrous and glandless" and "hairy with normal glands," fed preferentially on the former and oviposited preferentially on the latter. General observations in experimental field plantings suggest that weevils tend to congregate preferentially on plants with normal green foliage when these occur in populations segregating for red and green color. All the characters mentioned—hairiness, absence of glands, red color—are controlled by known genes whose inheritance is relatively simple. Two incompletely dominant genes govern hairiness, four recessives—absence of glands, and a single incompletely dominant—red color. It has been possible to extract lines homozygous for the six genes concerned. If the red, hairy glandless combination were of sufficient economic worth, it might be possible to transfer it to a commercial variety. This, however, would almost certainly be a long-term proposition, not justified unless the particular combination of characters were shown to confer a considerable degree of resistance to boll weevil attack.

Two types of study are reported in this paper. The first represents a continuation of earlier laboratory tests (Stephens 1959). These are simple experiments, with small numbers of insects and carried out under highly artificial conditions. They should be regarded merely as a preliminary screening device for picking up any large differences which may exist in the particular strains tested. The second study is concerned with a small field experiment in which an attempt was made to compare the amounts and types of damage inflicted by boll weevils on the following strains:

N	"Normal" (standard Upland variety)
H	Hairy
HG	Hairy-glandless
HGR	Hairy-glandless-red

A test of this kind has two requirements: (1) that the initial level of infestation by the boll weevil be controlled, (2) that the strains tested be similar in earliness and potential productivity. Otherwise differences between

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[2] On leave of absence as International Cooperation Administration Fellow at North Carolina State College, 1959–61.

Table 1.—Summary of laboratory tests comparing feeding and oviposition preferences of boll weevils when offered a mixture of buds from red and nonred strains.

Material Tested	No. of Tests	No. of Punctures in		No. of Ovipositions	
		Red	Other	Red	Other
Red vs. Green					
Egyptian hybrid segregating sibs	8	168	170	36	46
Upland varieties	11	191	240	68	69
Red vs. Yellow					
Upland hybrid segregating sibs	16	417	374	41	34
	8[a]	158	179	36	35
Total	43	934	963	181	184

[a] Mexican weevils substituted for local weevils in these tests.

strains in respect to number of buds available during the test period are confounded with differences associated with the characters under study. The first requirement was satisfied in part by conducting the test in isolation from other cotton crops in an area which had not grown cotton in the previous season; by surrounding each replication with a barrier of sorghum; and by introducing into each replication a standard number of adult weevils. The second requirement could not be satisfied with the material available. As expected, the standard Upland variety was considerably more productive than the recently synthesized mutant strains. A more serious effect which had not been anticipated was the irregular flowering and overall lateness of the HG and HGR strains. This led to sampling problems and certain difficulties in interpreting the data.

Laboratory Tests.—All tests were based on a standard procedure. The bracteoles were removed from eight buds of each of the strains to be tested, the buds in each set being matched for size. The buds from each strain were mixed and placed together in 1-pint Mason jars with wire mesh tops; and eight adult weevils, previously fed on buds of a standard commercial variety, were added to the jar. After an interval of 16 to 24 hours the buds were scored for total punctures and then carefully dissected and searched for eggs. The total numbers of punctures received and eggs laid in each set of eight buds were recorded.

Most of the weevils used were reared from fallen squares gathered in local cotton fields and incubated in the laboratory. In two small series of tests a strain of Mexican (nondiapausing) weevils, reared in the laboratory and kindly supplied by Dr. R. T. Gast, was used.

Table 1 summarizes briefly the results of 43 tests in which the weevils were offered a "choice" between the buds of red and either green (normal) or virescent yellow strains. (The character, virescent yellow, is determined by a simple recessive mutant, and as the name implies, interferes with normal chlorophyll development in all green parts of the plant, including the flower buds.) No significant preference was detected either for feeding or egg-laying. These results do not necessarily conflict with the field observation that red plants tend to be avoided (or overlooked) by the weevil. The finding of a red plant in the field may depend on visual stimuli—under the laboratory conditions the weevils were placed directly in contact with the buds and the visual reaction would be unimportant. The laboratory tests *do* indicate that red buds, when found, are not rejected by the weevil.

Table 2 summarizes the results of similar tests in which the weevils' reactions to buds of normal and glandless strains were compared. In two series of tests this comparison was superimposed on a comparison between the hairy and nonhairy characters. In the third series the hairy character was held constant. All the lines tested originated as segregates from a cross between Strain #6 (Glandless) and Strain #50 (Hairy) whose origin was reported in an earlier paper (Stephens 1959). As shown in table 2 the first series of tests, in which Mexican weevils were used, gave no evidence of preference. The second series, involving local weevils, showed significant preferences for feeding and oviposition on the nonhairy combinations. There was no significant difference between normal and glandless combinations. In the third series where normal and glandless were both combined with hairy, there were significant preferences for feeding and oviposition on the normal types. Taken at face value these results suggest that Mexican and local strains of weevils may react differently to the characters tested. They indicate also that for local weevils, nonhairy buds are the preferred sites for feeding and oviposition. Only when weevils are confined to hairy buds is a preference for normal over glandless types exhibited. These effects, if real, imply that the probable results of "multiple choice" experiments cannot be extrapolated from those obtained in "restricted choice" experiments, nor *vice versa*.

Field Study.—Plants of the control variety, N, and of the three mutant strains, H, HG and HGR were raised in the greenhouse. They were transplanted at the four-leaf stage to the experimental site. This was located at least 2 miles from the nearest cotton field in an area which had not grown cotton in the previous two seasons. The site was planted with sorghum: at transplanting time, the sorghum was uprooted from the future experimental plots and replaced by cotton planted in the same rows. Each plot consisted of four rows, one row for each strain, in random order. There were eight plants in each row approximately 18 inches apart with 42 inches between rows. Each plot was replicated seven times, with each replication separated from its neighbor by an equivalent area of sorghum.

The N variety commenced flowering 3 weeks earlier than the earliest of the mutant strains, H. A week later the HG and HGR strains commenced flowering but there was considerable variation in time of flowering between plants of the same strain. This large variation in

Table 2.—Summary of laboratory tests comparing feeding and oviposition preferences of boll weevils when offered a mixture of buds from strains combining hairy and glandless characteristics.[a]

No. of Tests	No. of Punctures in				L.S.D. (0.05)	No. of Ovipositions				L.S.D. (0.05)
	Hairy		Nonhairy			Hairy		Nonhairy		
	N	G	N	G		N	G	N	G	
8[b]	103	109	110	140	n.s.	35	22	32	47	n.s.
8	81	75	152	133	48.6	23	21	30	47	13.1
20	477	322	—	—	95.7	165	129	—	—	33.9

[a] N = normal (with glands); G = glandless.
[b] Mexican weevils substituted for local weevils in these tests.

earliness was unexpected, and an attempt was made to correct it by daily removal of flowers from all strains until the latest row had produced a flower. This occurred at the end of July 1960, at which time the plots were free of boll weevils. On August 1 six adult weevils were placed in the middle of each row. Each group of six was enclosed in a plugged glass vial which was pushed into the soil in the middle of each row. When all were in place, the weevils were released by unplugging the vials. On the following day, a few weevils found dead in the vials were replaced by fresh ones. All weevils were kindly provided by Dr. R. T. Gast from a strain originating in Alabama which had been reared in his laboratory for several generations.

Scoring commenced on August 4th and was continued at 2- to 3-day intervals, depending on weather conditions, until a total of 10 scorings had been made. Finally, a "strip count" was made, all flower buds and bolls being removed from the plants.

Method of Scoring.—The usual method of sampling for boll weevil damage is to collect flower buds at random from each plot. In small plots it is very difficult to collect a random yet representative sample of buds in this way. In the present experiment a method of selective sampling was adopted. A single bud was collected from each plant. This was chosen as the penultimate bud on the third flowering branch (sympodium) counting downward from the apex of the main stem. If the bud in this position was missing (because of natural shedding or previous collection) the bud from the same position chosen successively from the fourth, fifth or sixth sympodium was collected in its place. Ideally this system should result in the sampling of buds of equivalent physiological ages and near the optimum size for oviposition. In practice it was not possible to fulfill the sampling conditions in the case of the HGR strain. Because of its late flowering and erratic bud production it was not possible to sample more than half the plants at any individual scoring date. Consequently, the incomplete data obtained from this strain were not included in the statistical analyses to be reported here. In the remaining strains sample size varied from a low of three buds per row (in HG) to a high of eight buds per row (complete sampling).

After collection the buds were brought into the laboratory and the numbers of punctures counted—no distinction being made between feeding and egg punctures. All buds were then dissected and counts of eggs and larvae recorded separately.

At the end of the experiment a final "strip count" was made, the numbers of buds and bolls per row being recorded. These were brought into the laboratory and scored for damage. Unpunctured buds were scored as "undamaged." The bolls were opened and scored as "undamaged" if inspection revealed no sign of internal injury.

Analysis of Data.—Because of the small samples obtained on certain scoring dates, the results of successive scorings were combined for statistical analysis. Thus the data obtained from each row represented the combined counts obtained in the 1st+2nd, 3rd+4th, . . . , 9th+10th scoring dates. Analyses of variance were then conducted on the basis of 3 strains×7 replications×5 combined scoring dates, and were applied to the following data:

(1) The percentage of damaged buds (i.e., with one or more punctures). The percentages were converted to arc sines for the purpose of statistical analysis.
(2) The average number of punctures per damaged bud.
(3) The average number of ovipositions (i.e., eggs plus larvae) per damaged bud.
(4) The average number of larvae per damaged bud.

Because of the range of counts from zero upward in (3) and (4) the square-root transformation ($\sqrt{x+1}$) was applied to the data before analysis. In the case of the final strip count, percentages were converted to arc sines prior to statistical analysis.

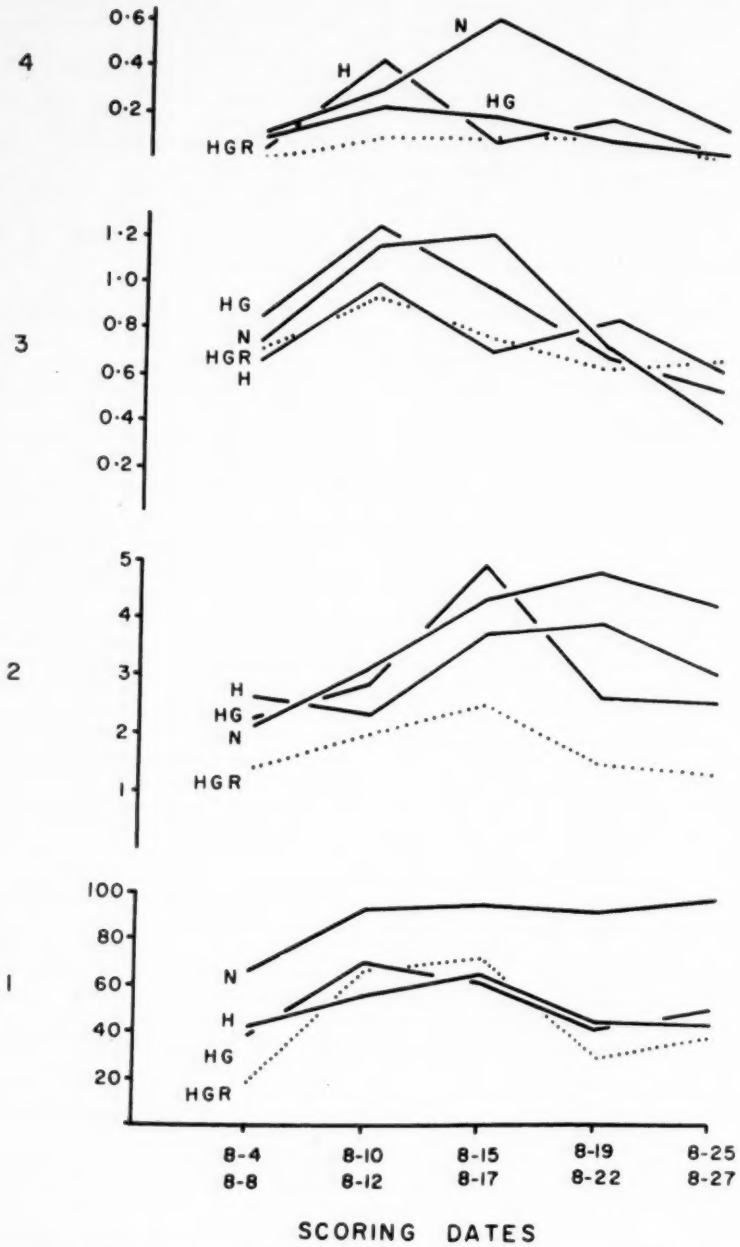
RESULTS.—The variance analysis for all data recorded (excluding the final strip count) is presented in abridged form in table 3. A split-plot analysis was used in which the term "Error (a)" was the interaction between replications×strains, and "Error (b)" the pooled interactions, strains×scoring dates and strains×scoring dates×replications. Mean squares associated with *replications* were low and insignificant except in the case of ovipositions per bud. Variations were expected to be small because of the precautions employed to insure a uniform initial infestation. No explanation can be offered for the differences between replications in respect of oviposition. Mean squares associated with *strains* were highly significant in respect of percentage buds damaged and number of larvae per bud. In the lower part of the table it may be noted that mean squares associated with scoring dates were in all cases highly significant—they merely reflect time trends in the activity and size of the boll weevil population.

The mean values for the three strains are compared in table 4. There were no significant differences between

Table 3.—Mean squares obtained in the analyses of variance of data as indicated below.[a]

SOURCE	D.F.	D	P	O	L
Replications	6	188.66	1.3361	0.0625*	0.0085
Strains	2	10841.26***	5.2050	0.0101	0.0697**
Error (a)	12	185.88	1.6505	0.0172	0.0058
Scoring dates	4	1110.68***	13.0993***	0.1597***	0.0533***
Scoring dates×strains	8	240.17*	3.3014*	0.0227	0.0205***
Error (b)	72	105.04	1.2476	0.0143	0.0044

[a] D—percentage of buds damaged (transformed data).
P—average number of punctures per damaged bud.
O—average number of ovipositions per damaged bud (transformed data).
L—average number of larvae per damaged bud (transformed data).
*** Significant at 0.1% level; ** Significant at 1% level; * Significant at 5% level.



FIGS. 1 to 4.—The amount of damage inflicted by boll-weevils on one standard variety and three mutant strains of Upland cotton.
N—Standard Upland variety.
H—Hairy strain.
HG—Hairy-glandless strain.
HGR—Hairy-glandless-red strain.

FIG. 1.—Percentage of buds damaged.
FIG. 2.—Average number of punctures per damaged bud.
FIG. 3.—Average number of ovipositions per damaged bud.
FIG. 4.—Average number of larvae per damaged bud.

the two mutant strains, H and HG, and it may be concluded that the presence or absence of glands had no effect under the conditions of this experiment. Both mutant strains had a lower percentage of buds damaged and a lower number of larvae per bud than the standard Upland variety, N. The numbers of punctures per bud were lower in the mutant strains than in the standard variety though the difference between strains was not significant in the analysis of variance (table 3).

There remain for consideration the interactions between strains and scoring dates which are given in the lower part of table 3. Only the interaction concerned with oviposition was insignificant. The interaction involving number of larvae per bud was highly significant. The nature of these interactions can best be appreciated from the graphic presentations in figures 1 through 4, where the amount of damage averaged over all replications is plotted against successive scoring dates for each strain separately. There was a general tendency for the mutant strains to receive less damage than the standard variety over all scoring dates, except in the case of the oviposition curves (fig. 3) in which the differences were insignificant. In the case of percentage buds damaged (fig. 1) it is clear that the interaction between strains and scoring dates only concerns the mutant strains. The standard variety, N, received considerably more damage than the mutant strains over all scoring dates. In the case of numbers of punctures per damaged bud (fig. 2) the amount of damage inflicted on the standard variety was exceeded significantly only by that inflicted on the mutant strain, HG, on one of the five scoring dates. There was a similar situation regarding number of larvae per bud (fig. 4). Over most of the scoring dates, buds of the standard variety contained more larvae than the buds of the mutant strains, but on one scoring date the buds of H gave a higher count.

The results obtained in the final strip count are given in table 5. The figures reflect the large differences between strains with respect to earliness. Thus the percentages in the last row of the table show that more than 50% of the total reproductive output (buds plus bolls) is represented as bolls in the N variety as compared with 14% in H and only 4% in HG. From the upper part of the table it can be seen that there were no significant differences in the number of buds per row, but that the percentage of damaged buds was more than twice as large in the N variety as in the mutant strains. Consequently there were many more buds surviving undamaged in the mutant strains than in the standard variety. In the lower part of the table the corresponding boll counts are presented. The boll set was very variable within strains so that only large inter-strain differences proved to be significant. The early flowering N variety had produced more than four times as many bolls as the next earliest mutant strain, H, which in turn had produced four times as many bolls as the latest flowering strain, HG. These large potential differences in boll production were almost obliterated by the fact that 90% of the bolls in the N variety were damaged as compared with 60% and 70% in HG and H, respectively.

It will be recalled that the inadequate samples obtained from the mutant strain, HGR, were not included in the foregoing analyses. Their mean values (averaged over all replications) have been plotted, however, with similar data from the other three strains in figures 1 through 4. There was an overall tendency for the HGR strain to receive less damage than the other strains in the test which may, or may not, be significant.

INTERPRETATION.—On the basis of earlier laboratory studies (Stephens 1959) it was expected that presence or absence of glands in the cotton plant might influence the amount of boll weevil damage in the field. Later studies, summarized in table 2, gave inconsistent results. When weevils were offered the "choice" of the four possible combinations of hairy and glandless buds, they preferred nonhairy buds for feeding and oviposition sites but did not discriminate between buds with normal glands and glandless buds. When the "choice" was limited to hairy normal and hairy glandless combinations, the former combination was preferred.

The field study reported here involved exactly the same strains as those tested in the laboratory. None of the data obtained in this study gave any indication that the weevils discriminated between presence or absence of glands. The main differences between strains were attributable solely to differences in hairiness. It remains possible, however, that had the field tests been confined to the hairy strains, H and HG, differences might have been detected—as they were in the laboratory tests.

Wessling (1958b) concluded that the partial resistance to boll weevil attack exhibited by his hairy lines was due to "nonpreference" (Painter 1951). He noted that the weevils were restless when attempting to feed on hairy plants, and also that they were not successful in attempting to enter squares which were "still small with the

Table 4.—Comparisons of strain means.[a]

CATEGORY	N	H	HG	L.S.D.
Percentage buds damaged	89.5 (76.4)	50.3 (45.2)	52.4 (46.7)	— (3.2)
Average number of punctures per damaged bud	3.7	3.1	3.0	0.30
Average number of ovipositions per damaged bud	0.83 (1.34)	0.75 (1.31)	0.83 (1.34)	— (0.03)
Average number of larvae per damaged bud	0.30 (1.14)	0.14 (1.06)	0.12 (1.05)	— (0.02)

[a] Transformed equivalents, when these were used in analysis, are given in parentheses below each mean.

Table 5.—Comparisons of the numbers of buds and bolls and their respective damage as determined from the final strip count (9-1-60).[a]

CATEGORY	N	H	HG	L.S.D. (0.05)
No. of buds per row	50.6	78.9	75.6	25.3
Percentage of buds damaged	94.0 (78.1)	39.6 (38.9)	54.6 (47.8)	— (7.3)
No. of undamaged buds per row	3.0	47.7	34.3	—
No. of bolls per row	53.7	12.4	3.0	18.1
Percentage of bolls damaged	90.1 (72.1)	67.0 (59.0)	59.7 (52.5)	— (31.1)
No. of undamaged bolls per row	5.3	4.1	1.2	—
Bolls as per cent of total i.e., buds plus bolls	51.5	13.6	3.8	—

[a] Transformed data are shown in parentheses below the original figures.

Table 6.—The effects of removing bracteoles from buds of hairy and nonhairy strains on boll-weevil damage.[a]

Category	With Bracteoles		Without Bracteoles		L.S.D (0.05)
	Hairy	Nonhairy	Hairy	Nonhairy	
No. of punctures	0.4 (1.15)	14.8 (3.92)	10.0 (3.17)	14.4 (3.84)	— (0.88)
No. of eggs laid	0.2 (1.08)	3.6 (1.95)	4.4 (2.18)	1.8 (1.63)	— (0.79)

[a] Results obtained from five tests. (Data transformed for analysis are shown in parentheses.)

bracts tightly closed." It now appears that the latter observation holds the clue to the major source of resistance offered by the hairy strains. Although the H, HG and HGR strains were derived from Wessling's original hairy lines, it has been possible to increase the degree of hairiness by selection. During the development of the squares in these strains, hairs lining the inner surfaces of the bracteoles become interlaced, effectively converting the tripartite whorl into a closed "capsule." It can be easily demonstrated that buds so protected are rarely damaged by the weevil. Table 6 presents the results of a small laboratory experiment in which weevils were offered the "choice" of hairy and nonhairy buds, with or without the bracteoles removed. The data show clearly that the bracteoles protect the hairy buds from feeding and oviposition but have no effect on the nonhairy buds. Unfortunately the sealed bracteole condition does not persist until the flower blooms, so that there is a vulnerable period of about 7 days prior to flowering in which the hairs are "unlocked" and the bracteoles flare open. Nevertheless the number of vulnerable buds present at any one time are reduced in the hairy strains and the level of damage can never reach 100%.

The protective effect of the sealed bracteole condition is well illustrated in figure 1. In the case of the standard variety, N, the percentage of damaged buds rises rapidly with time and plateaus just short of 100%. In the hairy strains, damage reaches a peak below 70% and then declines. It is believed that this difference in behavior is due primarily to the sealed bracteole condition in the hairy strains for two reasons: (1) At the time of collection it was noted that the bracteoles surrounding hairy buds were often sealed, and that in this condition the buds were rarely punctured. (2) As shown in figure 1, damage to the standard variety, N, reached almost 100%. If the partial resistance of the hairy strains were a matter of feeding preference only, it would be expected that the virtual saturation of feeding sites on N at this time would have forced the weevils to transfer to H and HG and the damage inflicted on these strains would have continued to rise. The fact that maximum damage was limited to 70% suggests that some form of mechanical protection was operating.

Although there were no significant differences in oviposition, as measured by the calculation, "average number of ovipositions per damaged bud," the number of larvae observed in the buds of the standard variety was significantly greater than in buds of the hairy strains. This difference was consistent at each scoring date, except in the case of H, where one early larval count exceeded that obtained in N (fig. 4). It seems likely that eggs hatched more slowly in the buds of the hairy strains—presumably an antibiotic effect not previously recorded. It is possible that the apparent antibiosis is quite independent of hairiness, and has been incorporated in the three hairy lines by chance. However, the independent genetic recombinations involved in the synthesis of these lines would make such a chance incorporation highly improbable, unless a single gene closely linked with one or other of the hairy genes were responsible for the antibiotic effect. An alternative possibility, worth future investigation, is that the apparent antibiosis is a secondary effect resulting from the physiological ages of the buds sampled. It has been known for some time (Hunter & Pierce 1912) that buds of intermediate size are preferred sites for oviposition. Possibly buds of optimal age for oviposition are also of optimal age for egg hatchability and larval development. If the sealed bracteole in hairy strains protects buds of optimal age from weevil attack, then oviposition might be restricted to older buds (with open bracteoles) "sub-optimal" for larval development. Clearly the method of selective sampling adopted in this experiment for the purpose of reducing variations in the physiological ages of buds would not be efficient for further comparative studies on antibiosis.

The incomplete data obtained from the HRG strain do not permit any definite conclusion to be drawn concerning its potential resistance to boll weevil attack. Figures 1, 2 and 4 suggest that the incorporation of the red character into a hairy strain might increase its resistance. On the other hand, none of the laboratory tests (table 1) indicated that weevils could discriminate between the buds of red and normal green strains when placed in contact with them. In the field it is possible that a "seeking preference" might operate (i.e., buds enclosed in red bracteoles on red plants might be located less easily).

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Dieldrin Residue on Vegetation in an Irrigated Pasture[1]

J. V. Smith,[2] J. C. Street,[3] G. R. Schultz,[2] and L. E. Harris[3]

Abstract

Levels and duration of dieldrin residues were studied on four dominant types of vegetation in a typical water-logged irrigated pasture following single applications of water emulsion and granular formulations of dieldrin. Dieldrin emulsion applications of 0.125, 0.25, 0.5, and 1.0 pound per acre and granular dosages of 0.5 and 1.0 pound per acre were made on various test plots in the study pasture. The pasture was irrigated by wild flooding four times during the season. Each plot was sampled for dieldrin analysis 0, 1, 2, 4, 6, 8, 12, and 16 weeks after treatment.

There appeared to be no marked differences in residue content among the four predominant pasture plant types for either formulation. With the granular applications, the detectable amount of dieldrin never exceeded 1.2 p.p.m. and persisted at low levels through 12 weeks. With emulsion treatments, typical persistence curves became established after 1 or 2 weeks of rapid degradation. Initial values of 6.8, 16.4, 59.0, and 115.0 p.p.m. for the four rates of application were found immediately after treatment, and residues of 0.3, 0.4, 0.5, and 1.1 p.p.m. were found at 16 weeks. Average dieldrin values for the control and reference plots never exceeded 0.2 p.p.m., the minimum detectable concentration. Likewise, flooding did not appear to influence the amounts of dieldrin residue. From the standpoint of mosquito control, granular formulations of dieldrin would be preferable for residual larviciding of pastures and hay meadows because of the much lower residues obtained without sacrificing larvicidal effectiveness.

Mosquito production on irrigated fields has been found to occur most often on those used for pastures, hay meadows, and other close-growing forage crops where water frequently ponds for extended periods. Dieldrin and several other chlorinated hydrocarbons continue to offer promise as larvicides for controlling mosquitoes associated with irrigation in a number of western areas where resistance to these insecticides has not developed. Field tests in the Milk River Valley, Montana, during 1955 and 1956, reported by Smith & Shultz (1957) and Shultz & Miles (1959), showed that single applications of water emulsion and granular formulations of dieldrin applied at 1.0 pound of toxicant per acre, were effective for an entire season in controlling mosquito production in habitats associated with irrigation. Miles & Shultz (1959) found that many of the 1956 treatments continued to be effective during the second season following application and that granular dieldrin applied in the spring of 1957 at the rate of 0.5 pound toxicant per acre provided effective control of mosquitoes throughout the season. A final report by Miles (1960) states that single preflood and postflood applications of dieldrin as water emulsions and granular formulations at approximately 1.0 pound toxicant per acre did not provide control of mosquitoes during the third season after treatment, and granular dieldrin at 0.5 lb./acre was not effective as a mosquito larvicide through the second season. He also showed that preflood applications of granular dieldrin at 0.25 lb./acre gave almost complete control throughout one season.

Under public law 518 (1954), a dieldrin residue tolerance of zero was established for most forage crops. Before dieldrin can be recommended as a residual larvicide on irrigated forage crops, information is needed regarding the amount and duration of dieldrin residue on vegetation following the application of various formulations of this insecticide.

Early investigations by Hopkins et al. (1952) on the persistence of insecticide residues on forage crops showed a loss greater than 98% of the original deposit in 21 days when one application was made. The insecticides tested were parathion, aldrin, lindane, BHC and DDT applied to alfalfa as a dust at the rate of 2 pounds of toxicant per acre. On a green weight basis, DDT residues persisted the longest, requiring about 40 days to give losses down to insignificant levels. Even when 6 pounds of toxicant per acre were applied (three treatments at the rate of 2 pounds of toxicant per acre), negligible levels of residue were found after 30 days. At this time and with the exception of DDT, these residues were in most cases less than 2 parts per million (p.p.m.).

Studies made by App & Carter (1956) included the treatment of two pastures with dieldrin granules, one at the rate of 0.53 pound and the other at the rate of 4.18 pounds of actual dieldrin per acre. At regular intervals after treatment, samples of the forage were taken for residue analysis. In the final samples taken 28 days after treatment, sufficient material had adhered to the foliage to show residues of 2.8 p.p.m. from the dosage of 0.53 pound per acre and 6.4 p.p.m. from the dosage of 4.18 pounds per acre.

Alfalfa treated at the rate of 0.25 pound of dieldrin per acre by Dogger & Bowery (1958) showed that dieldrin residues of more than 0.1 p.p.m. may persist 30 days after application as a spray, and detectable amounts of dieldrin (0.87 p.p.m.) may persist 15 days after application as a dust.

The present study was carried out during the summer of 1957 to determine the levels and duration of dieldrin residues on vegetation in an irrigated pasture following single applications of various amounts of water emulsion and granular formulations of dieldrin at dosages currently being used for residual larviciding to control mosquito production.

Procedure.—An irrigated pasture on a waterlogged tract of land about 6 miles northwest of Logan, Utah, was chosen for the study. A survey of the vegetation made during the latter part of May showed that the four predominant plant types were *Carex* sp. (sedge), *Eleo-*

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[2] Encephalitis Section, Technology Branch, Communicable Disease Center, Public Health Service, U. S. Department of Health, Education, and Welfare, Greeley, Colorado.

[3] Department of Animal Husbandry, Utah State University, Logan.

charis sp. (spikerush), *Juncus* sp. (rush), and *Distichlis* sp. (saltgrass), each occurring in more or less distinct, single-species stands at a number of locations on the study area.

In early June, 12 experimental plots for each of the four predominant types of vegetation were established on a 12-acre section of the pasture. Eight of these plots (two for each type of vegetation) were located on the upper side of the study area and were used as untreated reference plots. Samples of vegetation from these plots were analyzed to provide base-line values for establishing the true levels of dieldrin residue resulting from treatment. Of the remaining 40 plots, 32 received a specified treatment with dieldrin and eight were left untreated as controls. The control plots, distributed among the treated plots, were used to determine whether irrigation and rain caused the dieldrin to move off the treated plots. All treated and control plots were selected randomly.

On June 11, dieldrin emulsion was applied to one plot of each plant type at rates of 0.5 and 1.0 pound of toxicant per acre, and granulated dieldrin was applied to one plot of each plant type at rates of 0.5 and 1.0 pound of toxicant per acre. On June 17, a second group of plots was treated with granulated dieldrin at the rates of 0.5 and 1.0 pound of toxicant per acre, and dieldrin emulsion was applied to one plot of each plant type at the rates of 0.125 and 0.25 pound of toxicant per acre.

The granulated dieldrin (2.5%), formulated on 8/20-mesh bentonite granules, was applied with a modified lawn fertilizer spreader mounted on bicycle wheels. The dieldrin water emulsions, prepared from a 15% concentrate, were applied with a small power sprayer mounted on top of the hopper of the granule spreader (fig. 1). The finished spray for all dosages was applied at the rate of 25 gallons per acre with a nozzle pressure of 40 p.s.i. All insecticide applications were made when there was no standing water on the ground surface and no evidence of moisture on the vegetation. Care was exercised in applying the insecticidal material to insure uniform distribution and to avoid overlapping. Four times during the study the pasture was irrigated by wild flooding. Although the ground surface was irregular, an attempt was made to cover the entire area with water during each irrigation. Usually about 5 days elapsed between the beginning of irrigation and the disappearance of most of the surface water. The amount of rainfall during the tests was recorded from a standard rain gauge located on the pasture study area.

Vegetation samples were collected from the reference, control, and treated plots on the day that dieldrin was applied and at intervals of 1, 2, 4, 6, 8, 12, and 16 weeks after treatment. Each time a plot was sampled, the vegetation on five randomly selected quadrats (2′×3′ each) was clipped off about 2 inches above the ground surface. Hedge clippers, operated by a flexible shaft connected to a small gasoline engine, were used for this pur-

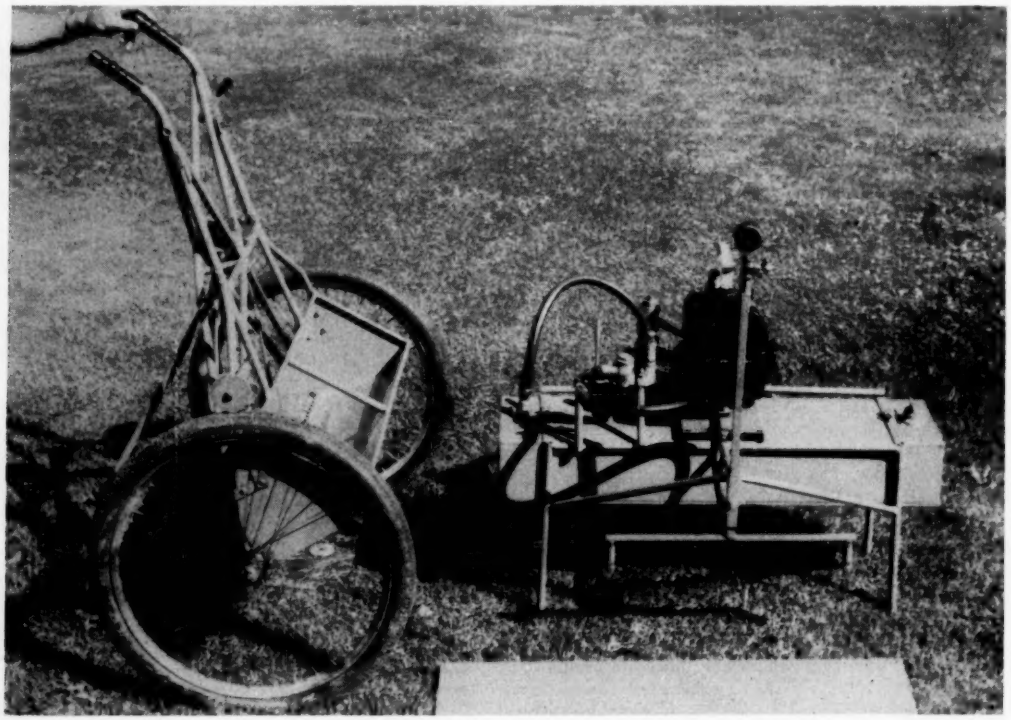

FIG. 1.—The modified lawn fertilizer spreader shown on the left was used for applying the dieldrin granules, and the small power sprayer on the right was used for applying the dieldrin emulsion. The sprayer was designed for mounting above the hopper of the spreader.

FIG. 2.—Hedge clippers, operated by a flexible shaft connected to a small gasoline engine, used to clip the vegetation.

pose (fig. 2). To avoid contamination of the untreated vegetation, separate clippers were used on the reference and control plots. No quadrat was clipped unless it had at least 50% of the ground surface covered by vegetation that, in turn, included at least 50% of the specified predominant plant type.

The vegetation from the five quadrats was weighed, then chopped into lengths of about 1 inch, and thoroughly mixed. Approximately 2 pounds of the mixed vegetation was placed in a plastic bag from which laboratory samples later were taken for a determination of dieldrin residue. All field samples were stored in an ice chest until taken to the laboratory, where they were kept frozen until analysis—in most cases, within a week after being clipped.

The phenylazide spectrophotometric method, essentially as described by O'Donnell et al. (1955), was used to determine the amount of dieldrin in 100-gram samples. The samples were extracted with 600 ml. of a 5% acetone in hexane solution; acetone was removed with water washes and the remaining extract concentrated for chromatography. The waxes that were not removed by chromatography on the magnesia-celite column were extracted from the solution containing the colored dieldrin product. Fortified samples were run daily to check on the recovery of dieldrin, which averaged 80%. Analyses were conducted on fresh material but all residue values were computed on a dry-weight basis.

The average amount of dieldrin found in samples of vegetation from the untreated reference plots never exceeded 0.2 p.p.m., the minimum detectable concentration. This is equivalent to about 0.1 p.p.m. on the fresh-weight basis.

RESULTS.—The average amount of dieldrin found throughout the season on samples of vegetation from plots treated with various dosages of emulsion and granular formulations is shown in figure 3. The values shown in this figure are averages for the four plant types that were treated. It will be noted that immediately after treatment, the dieldrin residue from the emulsion was many times greater than that from the granular formulations at the same dosages.

The average initial residue values on a dry-weight basis for the emulsion treatment ranged from 6.8 p.p.m. for a dosage of 0.125 pound per acre to 115 p.p.m. for 1 pound per acre. After 1 or 2 weeks of rapid degradation, typical persistence curves became established for the remainder of the season. Sixteen weeks after treatment the average residue values for the four dosages (0.125, 0.25, 0.5, and 1.0 pound per acre) were 0.3, 0.4, 0.5, and 1.1 p.p.m., respectively. At any time during the period of study, these residues were generally higher than those obtained from comparable dosages of granular formulations.

Average dieldrin residue values for the granular formulations during a 12-week period following treatment ranged from 0.2 to 0.4 p.p.m. for the dosage of 0.5 pound per acre and from 0.3 to 0.6 p.p.m. for the dosage of 1 pound per acre (fig. 3). The low residues found on the day of treatment show that few of the dieldrin granules lodged on the vegetation. The amount of dieldrin found in individual samples collected throughout the study varied widely but the maximum value never exceeded 1.2 p.p.m. The erratic variation in the amount of dieldrin in individual samples probably was due to the variation in the number of granules that lodged on the plants. After 16 weeks, the residual dieldrin for both dosages had dropped below 0.2 p.p.m., the minimum detectable concentration.

The amount of residue found throughout the season on each of the four plant types treated with emulsion and granular formulations at a dosage of 1 pound of dieldrin per acre is shown in figure 4. The values shown for the

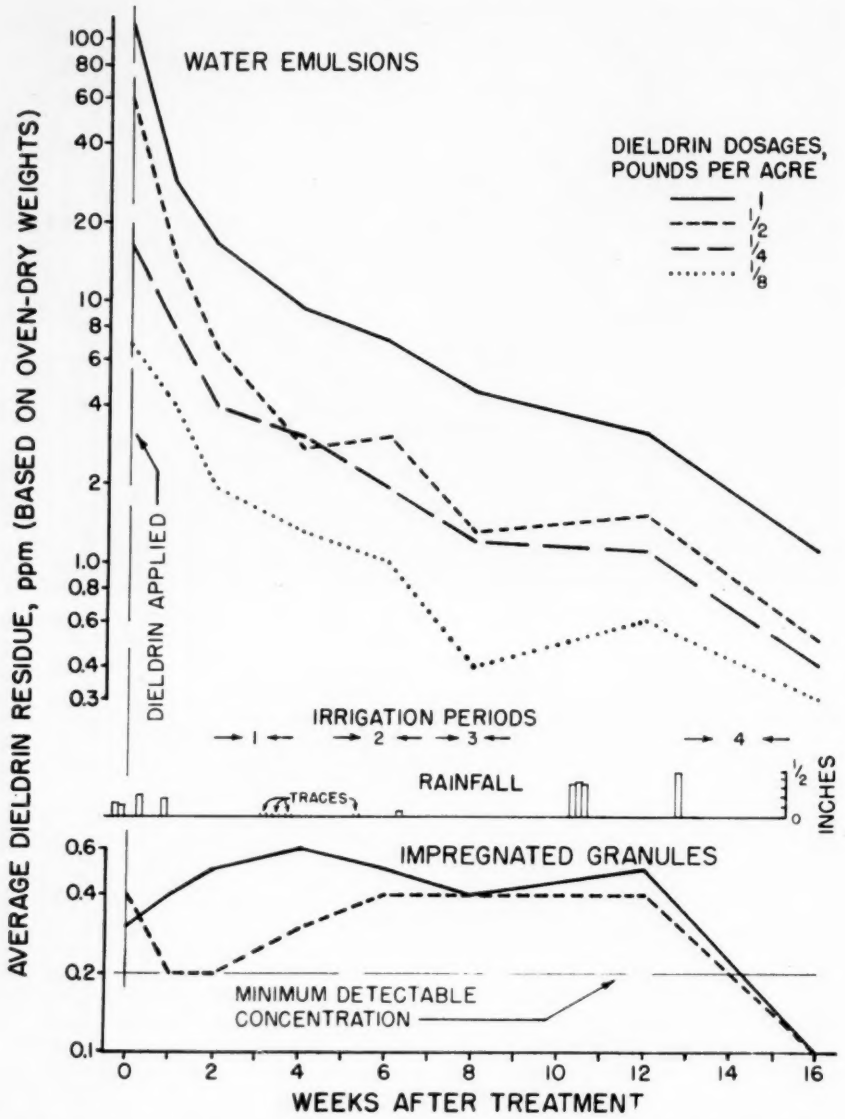

FIG. 3.—Comparative residues on vegetation from residual larviciding of irrigated pasture with emulsion and granular formulations of dieldrin.

granular formulations are averages for the two treated plots for each plant type. The pattern of the curves for the 0.5-pound dosage for both formulations for the four plant types was almost identical with that for the 1-pound applications. There appeared to be no major differences in the residues obtained with each of the four plant types except that on *Carex* sp. (sedge) they averaged slightly higher than on the other three plant types. This was true for both the emulsion and granular formulations at dosages of 0.5 and 1.0 pound of toxicant per acre.

The duration of the four irrigations and the daily rainfall during the period of study are shown in figure 3. There was no indication that irrigation or rain had any major influence on the dieldrin residue found on vegetation from plots treated with any of the formulations used. The average residue values for vegetation samples from the untreated plots remained essentially well below 0.2 p.p.m., the minimum accurately detectable concentration of dieldrin on a dry-weight basis. Flooding caused little or no movement of dieldrin from the treated to the untreated plots, despite their proximity.

DISCUSSION OF RESIDUE OBTAINED.—Hopkins et al. (1952) point out that two factors, weathering and growth, are primarily responsible for the loss of insecticide resi-

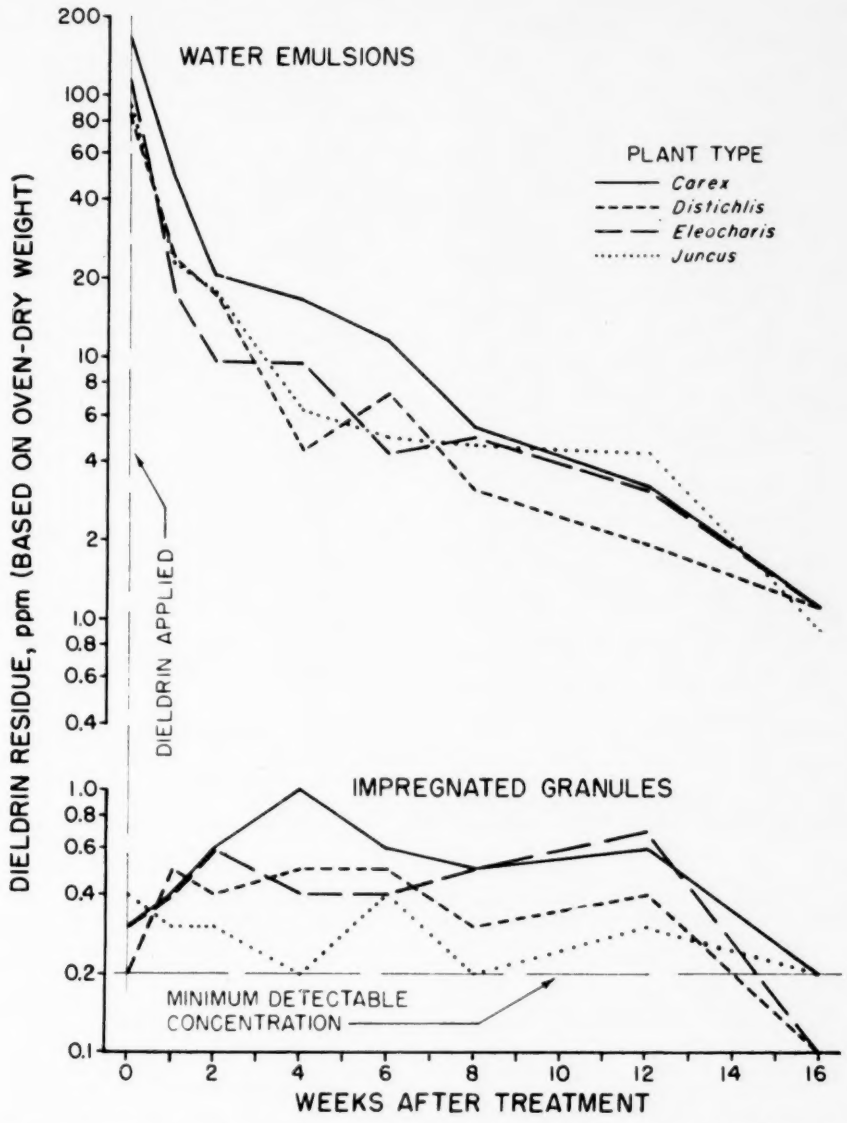

FIG. 4.—Comparative residues obtained on four plant types treated with dieldrin emulsion and granules at the rate of 1.0 pound of toxicant per acre.

dues on crops. Although growth does not result in a direct loss of residue, the result is an increase in plant weight or area in relation to the amount of residue. These workers indicate that a third factor, the volatility of the insecticide used, may be important and give data which indicate that this possibility should not be ignored in an overall evaluation of residue loss. Although these factors were not under investigation in this study, apparently they were operative and were responsible for some of the residue loss from the pasture vegetation treated with emulsion formulations of dieldrin. It also appears that, in general, each dosage of dieldrin spray responded similarly and somewhat proportionately to the factors responsible for residue loss during the 16-week period.

The persistence of residue detected on vegetation treated with granulated dieldrin is quite different from that shown for the emulsion formulations (fig. 3). Low detectable residue concentrations were persistent from the 1st through the 12th week, ranging only from 0.2 to 0.6 p.p.m. This condition is known to be accounted for partially by the lodging of a few impregnated granules on the vegetation. It is also important to consider translocation as a factor in maintaining a residue of insecticide on vegetation. Terriere & Ingalsbe (1953) showed that soil

treated with dieldrin at the rate of 10 pounds of toxicant per 6-inch acre contained residues as high as 5.5 p.p.m. during the third year after treatment. By bioassay with mosquito larvae, it was shown that potatoes grown in the plots during the third season after treatment contained residues with a toxicity equivalent to 0.02 p.p.m. of dieldrin. Other studies by Haines (1956), Lilly & Fahey (1956), Starnes (1950), and Ware (1959) showed that translocation was an important factor in the persistence of residues of BHC and lindane in forage and root crops. On this basis, it is quite possible that translocation could account in part for the maintenance of low-level residues of granulated dieldrin for 12 weeks. However, this has not been tested and therefore remains only as a possible answer for the persistence curves obtained.

In summary, the results of these investigations indicate that granular formulations of dieldrin would be preferable to sprays for mosquito control because of the low residues obtained without sacrificing larvicidal effectiveness.

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Imported Fire Ant Toxic Bait Studies: The Evaluation of Various Food Materials[1]

C. S. LOFGREN, F. J. BARTLETT, and C. E. STRINGER,
Plant Pest Control Division, Agric. Res. Serv., U.S.D.A., Gulfport, Mississippi

ABSTRACT

Laboratory tests with the imported fire ant, *Solenopsis saevissima richteri* Forel, showed that they would eat a wide variety of foods, including fats, proteins and carbohydrates. Dry foods, or foods that contained a minimum amount of liquid, showed little attractiveness. Field tests with individual imported fire ant colonies employing the dyed food technique indicated that various mixtures of vegetable or fish oils and flour, meals or dried blood were very attractive. Porous breakfast cereals impregnated with oils showed promise as baits which would be dry to handle. In small plot tests the following baits when combined with Kepone® (decachlorooctahydro-1,3,4-metheno-2H-cyclobuta [cd] pentalen-2-one) gave more than 90% control of imported fire ants: peanut meal plus peanut oil, peanut oil and monoglycerides, peanut butter, flour plus soybean oil, cotton seed oil or peanut oil, whole wheat flour and peanut oil, and fish oil plus dried blood.

Several investigators have studied the food habits of the imported fire ant, *Solenopsis saevissima richteri* Forel, in the field (Wilson & Eads 1949, U. S. Dept Agric. 1958, and Hays & Hays 1959). While there are some apparent contradictions in the observations reported, it may be concluded that this insect is an omnivorous feeder. Hays & Arant (1960) conducted extensive studies in the laboratory. From these tests they found tankage, fish meal, ground insect bodies, processed vegetable oils, digested corn and soybean protein plus fat, peanut butter and certain other mixtures high in protein and/or fat to be the most attractive food materials. They also found peanut butter and Kepone® (decachlorooctahydro-1,3,4-metheno-2H-cyclobuta [cd] pentalen-2-one) at a concentration of 0.125% and packed in soda straws to be a very good imported fire ant bait. Bartlett & Lofgren (1961) reported that vegetable oils and animal oils were very attractive foods. In addition they showed that peanut meal-peanut oil and Kepone (0.25%) was an effective imported fire ant bait.

In 1957 studies were begun to determine which types of food would be most acceptable for use in a toxic bait.

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This paper presents the results of laboratory and field tests conducted in these studies.

LABORATORY TEST PROCEDURES.—The tests were conducted with captive imported fire ant colonies. Ant colonies and their mounds were dug up in the field, placed in large wash tubs and removed to the greenhouse. The ants were kept from escaping either by placing the tubs in large, shallow pans filled with water, thus creating a moat around the tubs, or by dusting the inner side of the tubs with talc. The colonies were fed for a week or more before they were used in any tests. The food consisted of insects, vegetable oils, dog food and 10% sucrose solution in water. This feeding period was necessary to decrease the activity of the ants and make them more selective in their food preference. Colonies which continued to feed very avidly or fed very sparingly were not used.

The test procedure was as follows: The acceptance of each candidate food material was compared with that of a standard, 10% sucrose solution in water. This food was used because in laboratory tests it is taken by the ants as readily as peanut oil, and because it is a chemically definable substance thus insuring a uniform standard. The foods were exposed to the ants on pieces of aluminum foil (1 in.×1 in.). The liquid foods were soaked on 1-inch squares of blotting paper and the paper placed on the foil. An effort was made to expose equal surface areas or feeding areas to the ants. After 5 minutes, the numbers of ants feeding on each food material were counted and recorded. After this, the ants were removed from the food, the positions of the foods on the mound reversed, and a second 5-minute test conducted. A total of eight replications were made with each material, two with each of four colonies. The acceptance ratio of each material to the standard was calculated as follows:

$$\frac{\text{No. of ants feeding on candidate food}}{\text{No. of ants feeding on standard food}} = \text{acceptance ratio}$$

RESULTS.—Two hundred and twenty-two foods or combinations of foods have been evaluated. Most of these foods were purchased in grocery stores and were selected so as to obtain information on as many types of foods as possible. A list of all those which gave an acceptance ratio of 0.75 or higher is contained in table 1. While the list of materials is quite diverse, it is evident that all the acceptable foods contain some liquid. This is very evident with a material such as yeast hydrolysate, which is completely unacceptable when dry, but is taken as readily as the standard when dissolved in water. Undoubtedly, the reason for this is that worker ants do not ingest solid food but extract the fluids.

Nontoxic Bait Studies in the Field.—Food materials which showed promise in the laboratory tests were tested next in the field on individual imported fire ant colonies. The method employed for the field evaluation was the same as that described by Bartlett & Lofgren (1961) and consisted of incorporating dye in the test food, offering it to the ant colony for 24 hours and then examining 100 worker ants for the presence of dye in the alimentary tract. The percentage of worker ants containing dye when compared with the standard indicates the attractiveness of the food. Six replications were made for each food. The standard material used in all of these tests was peanut butter. It contains approximately 50% oil.

One of the big difficulties associated with a bait is to protect it from high amounts of rainfall. For this reason, of all materials listed in table 1, those considered most promising for field use were those containing oil. Field observations have shown that a material such as peanut butter will lose some of its oil during a rainfall but this does not appear to affect its attractiveness. During 1960 a series of tests was conducted to evaluate various combinations of vegetable oils, fish oil (Menhaden) or tallow, with some flours, meals or dried blood. The results are presented in table 2.

Table 1.—A list of food materials which gave an acceptance ratio of 0.75 or higher in laboratory tests.

Anchovy filet	Fish oil
Anchovy paste	Honey, diluted with water
Apple Sauce	Insects, freshly killed
Baby foods	Lard
Chicken with broth	Meat and bones, fresh
Egg yolks and bacon	Meat and bones, cooked
Ham and broth	Meat, potted
Lamb flavored with mint	Neat's-foot oil
Liver and broth	Peanut butter
Strained bananas	Recovered soy fatty acids
Strained fruit dessert	Salad dressing
Strained pears	Sardines, canned
Bean dip	Soft drink syrups
Blood hydrolysate (1:10 ratio with water)	Soybean oil
Casein hydrolysate (1:10 ratio with water)	Tallow
Cat food, canned, ground	Yeast hydrolysate (1:10 ratio with water)
Chocolate syrup	A. E. Staley Co. SPIB FA-19 enzyme digested corn proteln, digestion variable
Cheese	A. E. Staley Co., SPIB FA-19A enzyme digested corn protein, digestion variable
Cheese spread	A. E. Staley Co., SPIB FA-19C enzyme digested corn protein, digestion variable
Cocktail meatballs	National Dairy Products Co., N-Z-amine type E (moistened with water)
Corn, creamed	National Dairy Products Co., N-Z-amine type YT (moistened with water)
Corn, whole kernel, in milk	National Dairy Products Co., N-Z-amine type A
Corn oil	National Dairy Products Co., Edamin (moistened with water)
Cottonseed oil	
Cranberry sauce	
Dog food, canned, ground	
Dextrose (1:1 ratio with water)	

Table 2.—Results of tests to determine the acceptability of various food materials combined with vegetable or animals oils to imported fire ants.[a]

Type of Food (%)	Oil (%)	Ants Containing Dye After 24 Hours (%)
Wet Mash or Slurry-Type Baits		
Series I		
Whole wheat flour, 58	Peanut, 42	86
Peanut meal, 53	Peanut, 47	84
Cottonseed meal, 61	Peanut, 39	82
Wheat shorts, 41	Peanut, 59	80
Corn meal, 65	Peanut, 35	77
White flour, 65	Peanut, 35	77[b]
Peanut butter (standard)	—	81
Series II		
Whole wheat flour, 57	Peanut, 43	73
White flour, 56	Cottonseed, 44	63
61	Fish, 39	61
58	Soybean, 42	57
55	Peanut, 45	55
Whole wheat flour, 58	Corn, 42	54
58	Fish, 42	49
White flour, 51	Corn, 49	49
Whole wheat flour, 59	Cottonseed, 41	35[b]
62	Soybean, 38	35
Peanut butter (standard)	—	65
Series III		
Fish meal, 60	Fish, 40	79
Bone meal, 51	Cottonseed, 49	71[b]
66	Peanut, 34	67[b]
68	Fish, 32	50
53	Tallow, 47	40
Peanut butter (standard)	—	70
Series IV		
Dried blood, 67	Fish, 33	79
62	Cottonseed, 38	67
65	Corn, 35	59
69	Peanut, 31	50
68	Soybean, 32	43
Peanut butter (standard)	—	54
Oil-Impregnated Breakfast Cereal Baits		
Series I[c]		
Cheerios, 39	Peanut, 56	87
Rice Krispies, 47	Peanut, 48	86
Puffed Rice, 21	Peanut, 74	82
Peanut butter (standard)	—	65
Series II		
Rice Krispies, 29	Peanut, 71	83
Cheerios, 29	Peanut, 71	79
Puffed Rice, 25	Peanut, 75	73
30	Cottonseed, 70	67
25	Soybean, 75	62
24	Corn, 76	59
29	Fish, 71	34
Peanut butter (standard)	—	76
Series III		
All Bran, 69	Peanut, 31	78
Bran 100%, 70	Peanut, 30	73
All Bran, 75	Cottonseed, 25	69
74	Fish, 26	61
Bran 100%, 74	Fish, 26	57
76	Cottonseed, 24	53
Peanut butter (standard)	—	78

[a] Average of six tests.

[b] One of the colonies moved during test period and results are based on five replicates.

[c] Five per cent monoglycerides added to the oil in each formulation.

A majority of the combinations tested were highly acceptable to the ants, thus indicating that this type of formulation shows good promise for use in a toxic bait.

Because the slurry-type baits in the previous tests are limited in application principally to open areas, an effort was made to develop a dry, granular bait. Early tests with oils formulated on nonfood particles such as clay granules were not encouraging because it was necessary to keep the formulation very oily to obtain acceptance. It appeared from these observations that it would be necessary to use some type of food granule. Therefore, a series of tests was conducted to evaluate various commercially manufactured breakfast cereals as carriers for the oil. It was found that some of these would absorb two to three times their weight in oil under vacuum and still remain dry on the surface. In these preliminary tests it was necessary to roll the cereals with dry clay granules to remove the excess oil. Later investigations have shown that the cereals can be impregnated very easily by heating the oil to 150° to 175° C. before it is poured or sprayed on the cereal granules. This procedure is similar to that used for making insecticide granules. Three series of tests were conducted with five different cereals and five oils. The names and contents of each cereal were as follows.[2]

Cheerios, marketed by General Mills, Inc., general offices, Minneapolis 26, Minn., and composed of oat flour, wheat starch, sugar, salt, Na Phosphate, Ca Carbonate, coloring, niacin, thiamine and riboflavin.

Rice Krispies, marketed by Kellogg Co., Battle Creek, Mich., and composed of toasted rice, sugar, salt, malt, flavoring, butylated hydroxytoluene, vitamin B_1, niacinamide and iron.

Puffed Rice, marketed by the Quaker Oats Co., Chicago, Ill., contains thiamin, niacin, and iron.

All Bran, marketed by the Kellogg Co., Battle Creek, Mich., consists of wheat bran, sugar, salt, malt flavoring, butylated hydroxytoluene, vitamins B_1 and D.

Bran 100%, marketed by the National Biscuit Co., Special Products Division, New York, New York, consists of bran, sugar, salt, malt extract, and fig and prune juices.

All of the combinations were taken readily by the ants with the exception of the formulations containing fish oil. (See table 2.)

[2] The use of this and other trade names does not constitute an endorsement of any such product by the U. S. Department of Agriculture.

Table 3.—Average results of three field tests with three different baits containing Kepone (0.125%) as the toxicant.

Bait Components (%)	Pre-treatment Count of Active Colonies	Per Cent Reduction in Active Colonies After Weeks Shown				
		1	2	4	8	16
Peanut meal, 71, peanut oil, 29	114	32	80	69	85	98
Peanut oil, 80, monoglyceride, 20	97	7	56	61	79	94
Peanut butter	80	1	27	42	73	97
—Check	82	7	17	2	39	65

Toxic Bait Studies in the Field.—Three experiments were carried out to evaluate food materials for use in toxic baits. In the first experiment an evaluation was made of two toxic baits which had been reported effective previously. They were (1) peanut butter and Kepone packed in soda straws, Hays & Arant (1960) and (2) peanut meal-peanut oil (5 to 3 ratio) and Kepone, Bartlett & Lofgren (1961). In addition a third bait, peanut oil-monoglyceride (4 to 1 ratio), which had shown promise in laboratory tests, was also tested. The addition of monoglycerides to peanut oil at concentrations of 10%, or more, results in a product with a consistency similar to that of lard or tallow.

Kepone, at a concentration of 0.125% by weight of total formulation, was the toxicant in all three baits. The test plots were 2 to 3 acres in size. Two replications were in pastures and one was in a lightly wooded area. The peanut butter was dispensed in soda straws cut into lengths of $2\frac{2}{3}$ inches. The peanut oil-monoglyceride bait was applied with a hand grease gun and the peanut meal-peanut oil bait was applied by hand. The application rate for the peanut butter and peanut oil baits was 6 pounds per acre and for the peanut meal-peanut oil bait, 20 pounds per acre. All formulations were put out at bait stations in a 5- by 10-foot grid pattern. Table 3 summarizes the results.

After 16 weeks the control by the three baits ranged from 94% to 98%. The speed of control was comparatively slow for a bait treatment. Usually the maximum effect is noted after 2 to 4 weeks. The slowness may have been due to the fact that the baits were applied in April 1960, when the average daily maximum temperature was only 78.2° F. Tests conducted in the fall of 1960 under similar temperature conditions have also shown a delayed action. There was a big reduction in active colonies on the check plots. This was probably the result of not having an untreated zone between the plots with the result that some of the check colonies fed on the bait. After 6 months, the plots had become reinfested with incipient imported fire ant colonies.

The second experiment was initiated to determine if additional peanut oil in the peanut butter and peanut oil-monoglyceride formulations would affect their acceptability to imported fire ants. The baits formulated were: peanut butter alone and at ratios of 1 to 10, and 1 to $2\frac{1}{2}$, with peanut oil and peanut oil-monoglyceride at ratios of 5 to 1, 10 to 1, and 20 to 1. Each of the baits contained 0.25% by weight of Kepone and 0.25% by weight of Calco oil blue dye. The tests were conducted on 3-acre plots in a Bahia grass pasture. The test was not replicated. The peanut butter-peanut oil (1 to $2\frac{1}{2}$ ratio) and peanut oil-monoglyceride (20 to 1 ratio) were applied with a compressor type hand sprayer without a nozzle at bait stations in a 5- by 10-foot grid pattern across the plot. The other formulations were packed in straws and applied in the same grid pattern. Twenty-four hours after application, 100 worker ants from each of 10 colonies in each plot were examined for dye. Table 4 shows the per cent reduction in active imported fire ant colonies after 1, 2, 4 and 8 weeks and the average percentage of ants examined which contained dye for each bait.

Complete control of imported fire ants was obtained with all of the bait formulations after 4 weeks. The number of workers accepting the bait after 24 hours varied from 41% to 64%. Any correlation between the degree of acceptance and the control obtained was not determinable because all formulations gave complete control. The landowner disked the plots after the 8-week count, thus making it impossible to obtain an accurate count after 16 weeks. However, general observations showed that the plots were completely reinfested with incipient imported fire ant colonies.

Experiment three consisted of four series of tests conducted during the summer of 1960 to determine the effectiveness of nine food materials combined with Kepone as toxic baits. In all the tests Kepone was used at a dosage of 0.125% by weight of total formulation. The baits tested were as follows: (1) peanut oil and white flour, (2) soybean oil and white flour, (3) cottonseed oil and white flour, (4) peanut oil and whole wheat flour, (5) fish oil and white flour, (6) fish oil and dried blood, (7) Cheerios breakfast cereal impregnated with peanut oil,

Table 4.—Results of tests with imported fire ants to determine the acceptance and control with peanut butter and peanut oil-monoglyceride baits containing various amounts of peanut oil, Kepone (0.25%) and Calco oil blue dye (0.25%).

Bait Components (%)	Pretreatment Count of Active Colonies	Worker Ants Containing Dye After 24 Hours[a] (%)	Per Cent Reduction in Active Colonies After Weeks Shown[b]			
			1	2	4	8
Peanut oil, 95.2, monoglyceride, 4.8	108	60	69	99	100	100
Peanut oil, 90.9, monoglyceride, 9.1	59	53	51	88	100	100
Peanut oil, 83.3, monoglyceride, 16.7	48	54	21	79	100	100
Peanut oil, 9.1, peanut butter, 90.9	79	41	66	100	100	100
Peanut oil, 28.5, peanut butter, 71.5	72	56	72	94	100	100
Peanut butter	64	64	73	95	100	100
—Check	74	—	27	42	45	47

[a] One hundred worker ants examined from each of 10 mounds.
[b] All plots were reinfested with incipient colonies after 16 weeks.

Table 5.—Average results of three field tests with baits consisting of miscellaneous food materials and Kepone (0.125%).

Bait Components (%)	Pretreatment Count of Active Colonies	Per Cent Reduction in Active Colonies After Weeks Shown				
		1	2	4	8	16
	Series I					
Peanut oil, 55, white flour, 45	26	56	81	94	90	58
Soybean oil, 42, white flour, 58	23	38	70	97	99	54
Cottonseed oil, 44, white flour, 56	14	62	81	83	95	60
Peanut oil 1, peanut butter, 99 (Standard)	22	62	92	92	94	80
—Check	15	28	17	39	28	17
	Series II					
Peanut oil, 43, whole wheat flour, 57	17	56	92	84	56	—
Fish oil, 39, white flour, 61	18	7	59	72	52	—
Peanut oil, 1, peanut butter, 99 (Standard)	17	74	96	88	72	—
—Check	16	10	19	13	0	—
	Series III					
Fish oil, 33, dried blood, 67	25	77	84	83	68	—
Peanut oil, 1, peanut butter, 99 (Standard)	29	87	83	76	76	—
Peanut oil, 1, peanut butter, 99 (No insecticide—check)	20	0	0	0	7	—
	Series IV					
Cheerios breakfast cereal, 29, impregnated with peanut oil, 71	18	29	73	86	69	78
Purina Dog Chow (finely ground)	22	3	36	9	11	—
Staley's Protein Bait No. 76[a]	44	0	8	8	30	—
Peanut oil 1, peanut butter 99	35	33[b]	32	54	89	91
—Check	29	3	7	3	29	0

[a] Material supplied by A. E. Staley Manufacturing Company, Decatur, Illinois, consisting of amino acids, oils and proteins.
[b] One-week count based on only two plots; remainder on three plots.

(8) Purina dog food, and (9) A. E. Staley's Protein Bait No. 76. Baits 1 through 6 were all slurry-type materials which simulate peanut butter. The breakfast cereal bait is comparable to a granular insecticide formulation. The last two baits are dry, powdery to granular materials, which contain only a small percentage of oil. Peanut butter with 1% by weight of peanut oil added was employed as the standard bait in each test series.

The plots were 1 acre in size and were arranged in either a checkerboard pattern so that the treated plots touched only on the corners or with an untreated buffer zone between the plots (60 feet or more). The tests were in triplicate. The slurry-type baits were applied with a specially designed jeep-mounted applicator. Essentially it consisted of a compressed-air-driven grease pump connected to a 15-foot boom with discharge orifices at 5-foot intervals. The flow rate was controlled by the orifice diameter, the length of the tubing leading to the discharge orifice and the air pressure into the pump. The bait flowed from the nozzles in large drops or in a coarse stream. The whole wheat flour-peanut oil and the dried blood-fish oil baits were too heterogeneous to pass through the grease pumps and therefore were applied by hand. All of the dry baits in series IV were put out by hand. They were applied in strips every 10 feet across the plot. The application rate with the slurry-type baits and the breakfast cereal-peanut oil bait was 6 pounds per acre. The dog food was applied at the rate of 20 pounds per acre and the Staley Protein Bait No. 76 at the rate of 15 pounds per acre.

Table 5 shows the results of these tests over a period of 8 to 16 weeks. The formulations in series I, peanut oil, soybean oil or cottonseed oil and white flour, gave reductions ranging from 90% to 99% after 8 weeks as compared with 94% for peanut butter. In series II and III peanut oil and whole wheat flour or dried blood and fish oil gave control comparable to the peanut butter standard. A reduction of 86% after 4 weeks was given by the breakfast cereal impregnated with peanut oil bait in series IV as compared with 91% after 16 weeks for peanut butter.

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Horn Fly Control by the Pour-on Technique Using Ruelene or Toxaphene[1]

WILLIAM M. ROGOFF and PAUL H. KOHLER,[2] *South Dakota Agricultural Experiment Station, Brookings*

ABSTRACT

Hereford yearling cattle naturally infested with horn flies (*Haematobia irritans* (L.)) were treated by the pour-on technique or full-coverage sprays. Treatment was applied to 13 to 14 animals in each of six balanced, adjacent pastures. The animals in three pastures received one treatment, and those in the other three pastures received the alternate treatment. Pour-on treatments consisted of application of 100 ml. of concentrated emulsion applied to the back in each instance by means of a dipper. Comparisons were made of Ruelene® (*O*-4-*tert*-butyl-2-chlorophenyl *O*-methyl methylphosphoramidate) as pour-on at 9.6 g./animal and as 0.25% spray, and of toxaphene as pour-on at 18 g./animal and as 0.5% spray. The pour-on procedure provided superior control in terms of absolute reduction of fly numbers and of length of time treatment remained effective. The toxaphene treatments were notably more effective than the Ruelene treatments by both application procedures. Erythrocyte cholinesterase levels were mildly depressed and were significantly lower among the Ruelene-pour-on animals than among the Ruelene-sprayed animals (82% vs. 88% of initial activity). No skin irritation was observed in any instance.

The horn fly, *Haematobia irritans* (L.), is a common and important pest of cattle. It is susceptible to a wide variety of residual insecticides and can be readily controlled by spray applications or by backrubbers. The fly breeds in fresh cattle droppings and as an adult spends most of its time, whether feeding or not, on cattle. It frequently moves about from the back to the belly and to various other parts of the animal during this resting, mating or feeding period. It is this habit of congregating on the backs of cattle that is largely responsible for the success of backrubbers, as described by Rogoff & Moxon (1952).

In 1960, the pour-on technique was introduced for the systemic control of cattle grubs (Rogoff & Kohler 1960). This technique consists of the application of a small quantity of concentrated insecticide to the backs of cattle. This application of as little as 100 ml. of a concentrated mix, using apparatus no more complicated than a dipper, raises the question of the applicability of this technique to horn fly control as well as to grub control. Furthermore, if this technique is applicable to horn fly control, the question is also raised as to its comparison with current standard treatments.

EXPERIMENTAL DESIGN.—These experiments were conducted on the Cottonwood Substation of the South Dakota Agricultural Experiment Station located in west central South Dakota. Available for this work were six pastures (covering $1\frac{1}{4}$ square miles) that had been operated for many years in connection with a grazing experiment (fig. 1). These pastures were subjected to varying levels of grazing intensity; pastures 1 and 4 (80 acres each) being heavily grazed, pastures 2 and 5 (133 acres each) being moderately grazed, and pastures 3 and 6 (183 acres each) being lightly grazed. In each pasture were 13 to 14 Hereford yearling calves, averaging 653 lb. each on July 1, that were naturally infested with horn flies.

Fly numbers were estimated by two types of counting procedures. The first type, accomplished in the field, consisted of examining the animals from a vehicle or on foot, using field glasses. All flies were counted on a side of each animal. The second counting procedure was observation of the animals, after roundup, in chutes. In general it was found that chute counts were more reliable than field counts for groups of animals, though less so for individual animals since the disturbed flies could move from one animal to the next. The ease of counting in the chutes, however, increased the accuracy of group counts and more than made up for the disadvantage of flies moving from animal to animal. Wherever the chute-count technique

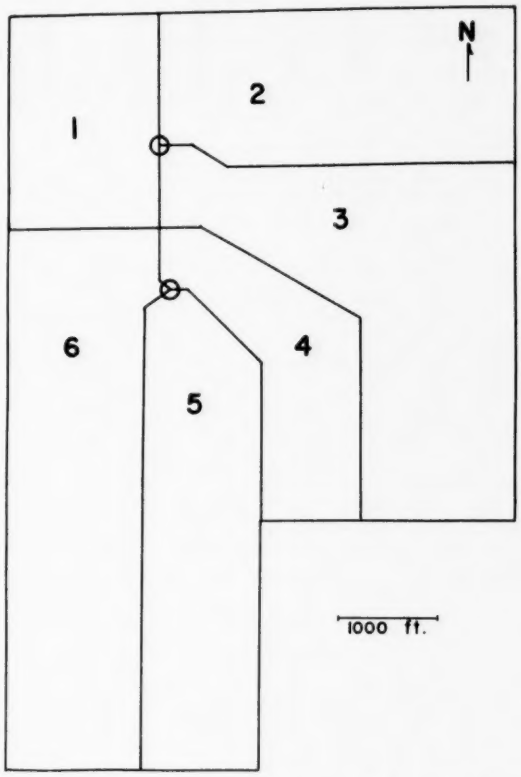

FIG. 1.—Layout of summer intensity grazing pastures, Cottonwood Substation. Area included in this plat, $1\frac{1}{4}$ square miles. Circles indicate common watering locations for each group of three pastures.

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[2] Entomologist and Associate Animal Husbandman, respectively, in the South Dakota Agricultural Experiment Station.

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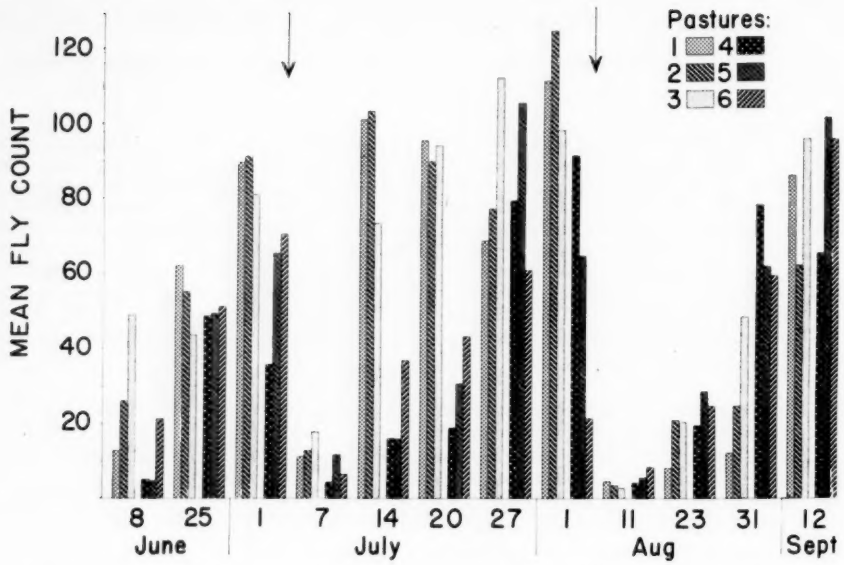

FIG. 2.—Horn fly counts obtained in each of six pastures on specified dates, 1960. Arrows indicate dates of treatment. See text for further explanation.

was utilized, animals from one pasture were kept separate from animals from other pastures.

The grazing experiment permitted utilization of the animals in pastures 1, 2, and 3 for one insecticidal treatment, and those in pastures 4, 5, and 6 for a second treatment. Two materials were used in the course of these experiments, Ruelene® (*O*-4-*tert*-butyl-2-chlorophenyl *O*-methyl methylphosphoramidate), and toxaphene. The Ruelene used was a 4 lb./gal. emulsifiable concentrate, applied either as a full-coverage spray or a pour-on. The spray consisted of a 0.25% mix utilizing 0.8 gallon or 7.2 g. of actual Ruelene per animal. The pour-on technique consisted of 20 ml. of the emulsifiable concentrate plus 80 ml. of water per animal (9.6 g. actual Ruelene each). The toxaphene used was a 6 lb./gal. emulsifiable concentrate, also applied as either a spray or pour-on. The spray consisted of a 0.5% mix applied to the animals until they were thoroughly wet. The pour-on consisted of 25 ml. of the emulsifiable concentrate plus 75 ml. of water per animal (18 g. of actual toxaphene each).

The Ruelene was applied to all animals on July 1. Animals in pastures 1, 2, and 3 were treated by the spray and those in pastures 4, 5, and 6 by the pour-on procedure. On August 1, toxaphene was used, with the methods of applications being reversed; that is, the animals in pastures 1, 2, and 3 were treated by the pour-on technique, whereas the animals in pastures 4, 5, and 6 were treated by the spray technique.

Physiological effects were investigated by means of red blood cell cholinesterase evaluations. The technique used was that of Michel (1949), except that red cell volume was corrected from average values rather than for each sample.

RESULTS.—Results of fly counts obtained are shown in figures 2 and 3. Figure 2 shows average counts for each pasture. Figure 3 shows average counts summed for each treatment, comparing the counts for pastures 1, 2, and 3 with those for pastures 4, 5, and 6. Chute counts were made June 8, July 1, July 14, August 1, and August 31. All other counts were pasture counts and represent flies per side of the animals.

Pretreatment counts are shown on June 8, June 25, and July 1. The counts of June 8 represent some residual control remaining from cable-type backrubbers that were still available but which had not been charged since the previous summer. Evidence of persisting effectiveness of these units was the presence of occasional dead horn flies on the backs of the animals.

The Ruelene tests commenced July 1 following the counts. Animals from pastures 1, 2, and 3 were sprayed, those in pastures 4, 5, and 6 were treated by the pour-on technique. Obvious reduction of horn flies on both groups of animals was shown on July 7, the spray group having dropped to 14, and the pour-on group to 7 flies per animal. By July 14, it is apparent that the spray treatment with Ruelene had failed (92 flies per animal) but that there was a persistance of effectiveness of the pour-on treatments at least to July 20, at which time fly numbers had risen to 31 per animal, but were still only one-third as high as the 90 per animal in the sprayed group.

Toxaphene tests were commenced on August 1, following the counts, as infestations had developed sufficiently in all pastures (in excess of 58 flies per animal in both treatments) to warrant the new phase of the experiment. In order to evaluate the obviously lower counts in pastures 4, 5, and 6 as compared with those in pastures 1, 2, and 3, prior to and following the Ruelene treatments, the animals that had been previously treated by the spray technique were treated by the pour-on method and *vice versa*. After the toxaphene treatments were applied, the

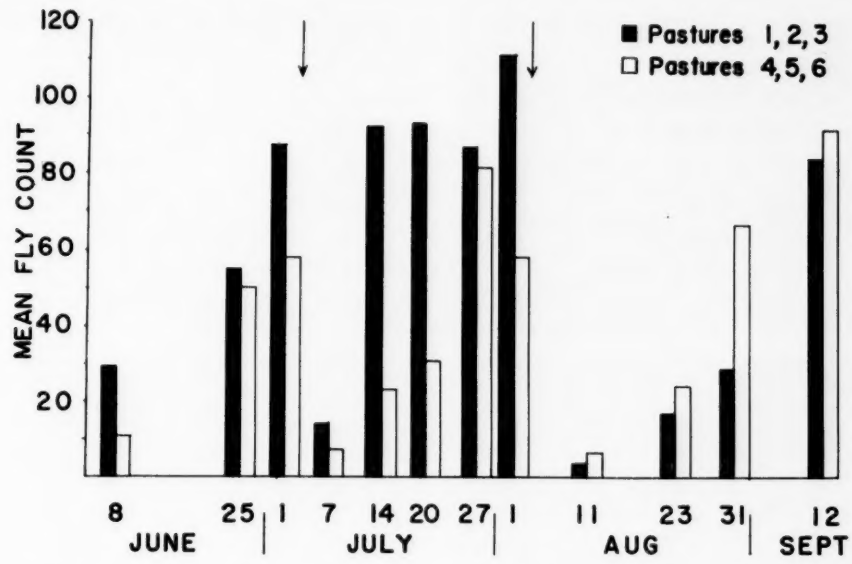

FIG. 3.—Horn fly counts obtained in pastures 1, 2, and 3 as compared with pastures 4, 5, and 6 on specified dates, 1960. Arrows indicate dates of treatment. See text for further explanation.

animals were returned to their original pastures. Thus the treatments consisted of pour-on applications for the cattle in pastures 1, 2, and 3 and spray applications for those in pastures 4, 5, and 6. Examination of figures 2 and 3 shows an obvious reduction of flies on all animals on August 11, the sprayed group having dropped to 6, and the pour-on group to 3.5 flies per animal. The persistence of the effects of the sprays lasted at least through August 23, at which time flies were still below 25 per animal. Persistence of pour-on effects with toxaphene extended at least to August 31, when counts averaged 29 as compared with more than 66 flies per animal in the sprayed group. It should be noted further that with the reversal of the method of treatment, there was also a reversal of fly abundance in pastures 1, 2, and 3 as compared with those in pastures 4, 5, and 6. Also, it should be noted that the toxaphene treatments, both spray and pour-on, provided effects that lasted considerably longer than the corresponding treatments with Ruelene, an effect more pronounced in the sprays than in the pour-on treatments.

Data on erythrocyte cholinesterase are shown in table 1. Cholinesterase activity was depressed in each group of animals with the greater degree of depression in the pour-on animals than in the sprayed animals. This difference (82% vs. 88% of pretreatment values) was significant at the 1% level. No skin irritation was seen in any of these tests.

DISCUSSION.—Data presented in this paper demonstrate that the pour-on technique is effective for horn fly control with either Ruelene or toxaphene. These data further indicate that the pour-on procedure is superior to the standard spray procedure. It should be realized that the application of sprays utilized only three-quarters of the total quantity of Ruelene used in the pour-on treatments. Direct comparison of quantities used is difficult, however, since some of the spray does not actually remain on the animal. This difference may explain the greater degree of cholinesterase depression among the pour-on treatments as compared with the spray treatments.

A reason for the greater effectiveness of the pour-on method as compared with the spray treatments might very well be the result of the much higher concentration of insecticidal material on a small area of skin following the pour-on procedure. Any possible increase in rate of absorption caused by higher concentration would tend to be overcome by the greatly reduced area in which absorption could take place. This same line of reasoning would apply to evaporation rates as well. This is substantiated by observations in the field that showed visual persistance of the residues on the backs of the cattle for several weeks following pour-on treatments. Since horn flies spend most

Table 1.—Bovine erythrocyte cholinesterase activity following spray and pour-on applications of Ruelene.

Pasture	No. of Head	Δ pH Units per Hour				Post-treatment Activity (% initial)
		June 8		July 14		
		Range	Avg.	Range	Avg.	
1	13	0.35–.56	0.46	0.30–.48	0.39	86
2	13	.35–.63	.49	.28–.58	.45	91
3	14	.36–.62	.48	.32–.57	.42	88
1, 2, 3[a]	40	.35–.63	.48	.28–.58	.42	88
4	13	.34–.66	.48	.34–.50	.40	84
5	13	.40–.63	.53	.33–.55	.43*	80
6	14	.37–.70	.50	.31–.59	.41	84
4, 5, 6[b]	40	.34–.70	.50	.31–.59	.41**	82**

[a] Spray treatment with 0.25% Ruelene, E.C., 0.8 gal./head, July 1, 1960.
[b] Pour-on treatment with 20 ml. Ruelene 4 lb./gal. E.C. and 80 ml. H_2O/head, July 1, 1960.
* Difference from pretreatment significant at 5% level.
** Differences from pretreatment in pastures 4, 5, and 6, and between treatment groups (1, 2, and 3 vs. 4, 5, and 6) significant at 1% level.

of their time on the cattle it is apparent that with the normal range of movement of these flies, there is sufficient time and opportunity for them to come in contact with the small reservoir of insecticide, hence providing control.

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A Flatid Planthopper, *Metcalfa pruinosa*[1]

H. A. DEAN and JACK C. BAILEY,[2]
Texas Agricultural Experiment Station, Weslaco

ABSTRACT

Observations on the life history of a flatid planthopper, *Metcalfa pruinosa* (Say), were made in the Lower Rio Grande Valley of Texas. Photographs of various stages are included. Eggs were found to hatch during March. Adults were first observed during May. Only one generation each year was found from 1956 through 1960. A dryinid parasite, *Psilodryinus typhlocybae* (Ashmead), was observed throughout this area.

A flatid planthopper, *Metcalfa pruinosa* (Say), has been observed on citrus for several years in the Lower Rio Grande Valley area, but its economic importance has been questionable for some time. Relative abundance of the insect was observed each year and chemical control was applied during years of greatest infestations in only a few cases. Investigations were begun in 1956 to study the life history for the purpose of developing efficient control measures for the insect.

Thomas Say (1830) originally described the species as *Flata pruinosa* from the United States. A comprehensive review of literature relating to this planthopper has been reported by Metcalf (1957). The insect has been reported from New York to Texas and Arizona; its range also includes several foreign countries. Most reports on the species are brief notes relating to host plants, ecology or abundance.

Information on control has been reported by a few authors. Walden (1922) found fewer individuals the following year after pruning grape vines back to one to two buds possibly owing to the destruction of the eggs. Wene (1950) reported good chemical control with 1.0% parathion dust or with a spray or dust of tetraethyl pyrophosphate.

Published reports were in the form of brief notes on the life history or the relation to other host plants. Howard (1903) reported that eggs were laid in a continuous raised slit in small sassafras twigs. Overwintering eggs hatched in May and nymphal development required three molts. The dirty yellow eggs were laid in September on the side and end to end. Walden (1922) reported the insect laid its eggs in long slits just under the bark of twigs in late summer or early fall.

LIFE HISTORY.—This planthopper overwinters in the egg stage in the area of Weslaco, Texas. Eggs were found in the bark of dead citrus twigs during January 1959. No particular oviposition pattern was evident; the eggs were found scattered singly in the bark. Measurement of 10 eggs averaged 0.80 mm. long by 0.38 mm. wide; with a range of 0.84 to 0.40 mm. Eggs began to hatch on March 12, 1959, and on March 23, 1960. A longer period of colder weather during the first 3 months of the year may have delayed the hatching during 1960.

First adults in the field were found 69 days after the hatching date during 1960. Dead twigs were removed from trees and placed in small cages covered with 52- × 52-mesh plastic screen. After hatching from the egg, nymphs failed to complete their development on 4- to 6-inch seedling grapefruit; however, a few adults developed in cages which contained seedling grapefruit 18 to 24 inches in height. Various stages of *M. pruinosa* are shown in figure 1.

The longevity of the adult stage has not been determined under local conditions although Wene (1950) reported the adult activity extending over a period of approximately 10 days. Factors influencing the duration of adult life may include the following: injury while being collected, confinement to cages, or unacceptable host plant growth in cages during confinement. Adults were observed in field trees during July and August 1957–1960, inclusive. Greater numbers were found during June than in the later months. During this period, adults were collected from the same groves week after week without the presence of nymphs. Such observations would indicate that locally adult activity may extend over a period of at least 2 months in this area. Only one generation was observed each year.

HOSTS.—Various hosts plants found in this area are reported because of their possible relation as a source of supply of the insect to infest citrus. It has been found more abundantly on grapefruit than on orange. Preference for grapefruit has been consistent where orange and grapefruit trees were growing adjacent to one another. Hackberry (*Celtis laevigata*) and anaqua (*Ehretia anacua*) trees (apparently the seeds are spread by birds) found beneath citrus served as hosts. One particular grove had an unusual number of these seedlings infested with a large number of planthoppers. Following the removal of these seedlings, the grove had very few planthoppers the following 2 years. Removal of hosts plants was considered

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[2] Thanks are due P. W. Oman and Karl V. Krombein of Insect Identification and Parasite Introduction Research Branch, Entomology Research Division, U. S. Department of Agriculture, for identification and verification of records.

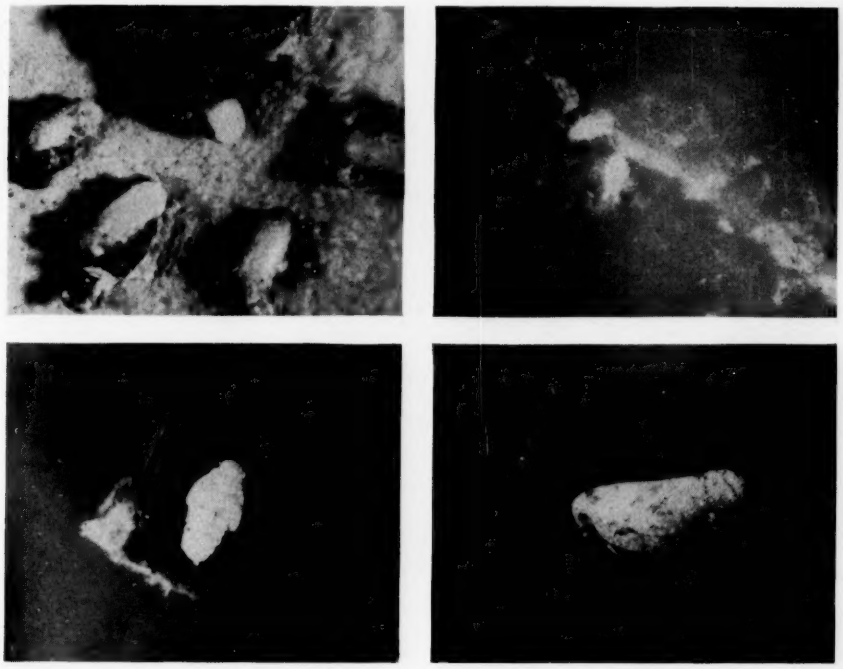

FIG. 1.—Various stages of *Metcalfa pruinosa*. Upper left—eggs. Upper right—young nymphs. Lower left—last nymphal instar. Lower right—adult.

a good cultural practice. Planthoppers were also found developing on purple nightshade growing beneath citrus trees.

Planthoppers were found in the nymphal stages on the following minor host plants: avocado (*Persea americana*), Rio Grande ash (*Fraxinus berlandierana*), fig (*Ficus carica*), pomegranate (*Punica granatum*), guava (*Psidium guajava*), papaya (*Carica papaya*), orchid tree (*Bauhinia variegata*), athel (*Tamarix articulata*), hibiscus (*Hibiscus cannabius*), jasmine (*Jasminum grandiflorum*), fiddle leaf fig (*Ficus lyrta*), ginger (*Albinia speciosa*), bougainvillea (*Bougainvillea spectabilis*), juniper (*Juniperus chinensis*), alamanda (*Allamanda cathartica*), Pyracantha (*Pyracantha crenulata*), Easter lily (*Lilium longiflorum*), corona vine (*Antigonon leptopus*), and devil's ivy (*Scindapus qureus*).

During these investigations a dryinid parasite, *Psilodryinus typhlocybae* (Ashmead), was found in almost every grove. These parasites developed to the prepupal stage during May or shortly thereafter and remained in this stage until the following December-January when pupation took place. In the laboratory, the larva though inactive for a considerable number of months, was found to move at times in the cocoon which was formed beneath the skin of the host. Male *typhlocybae* were smaller than the females. It appeared that the male either attacked younger nymphs or retarded planthopper development. Adults emerged during late December and in January. Figure 2 shows two female and one male *typhlocybae* prepupal larvae. It is not known whether there is a spring generation of the parasite prior to the generation with the long-cycle larvae.

Psilodryinus typhlocybae was originally described as *Labeo typhlocybae* by Ashmead in 1893 from specimens reared July 23, 1883, from a homopteron on *Celtis* and elm by C. V. Riley at Washington, D. C. Swezey (1903) found the larva encased in a felt-like sack protruding from a spiracle at the base of the abdomen of its host, and as the latter matured, the parasite was partially covered by the wing pads. The larva formed a cocoon beneath the remains of the host on the leaf. Also, it was indicated that in natural situations among the fallen leaves, the larva transformed to a pupa the following spring and the adult would emerge the latter part of the spring or early summer. These observations were reported for the parasite on another flatid planthopper, *Anormonis septentrionalis* (Spin.), in the northern part of the United States.

VIRUS TRANSMISSION TESTS.—Yamada & Sawamura (1953) reported a flatid planthopper, *Geisha distinctissima* (Walker), as a vector of a virus disease of satsuma orange. These authors also reported that *distinctissima* laid its eggs in dead twigs and advised the removal of twigs in order to reduce the population.

The possibility that *M. pruinosa* might serve as a vector of tristeza virus disease was considered during the course of this investigation during 1958. Infected Mexican lime seedlings (severe strain of tristeza virus as reported by Olson, 1956) were placed in 2- ×2- ×3-foot cages covered with 52- ×52-mesh plastic screen. Adult *pruinosa* were collected in 32-mm. diameter lusteroid tubes from

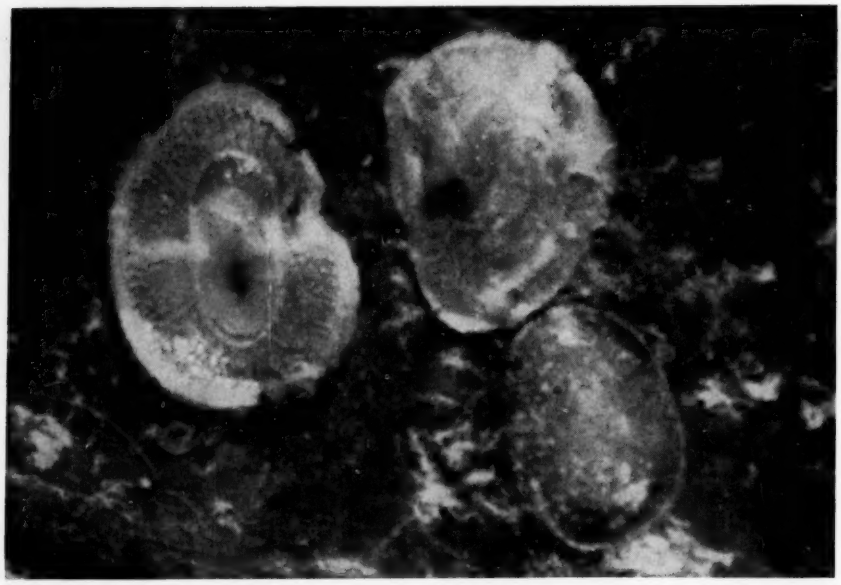

FIG. 2.—Prepupal larvae of *Psilodryinus typhlocybae*.

field trees. Each cage received 30 adults on June 13. Most of the adults were dead the following day when noninfected lime seedlings were placed in the cages, probably because of injury while being collected. Subsequently, 30 adults were placed in each cage about every 5 days on four subsequent dates. No positive transmission of the disease was found in six of the cages which held infected plants or in the two cages with noninfected plants. The difficulty experienced in handling the adults from the field suggested the possible use of nymphs instead of adults. Leaves and twigs infested with the planthoppers were pruned and placed in 1-gallon cylindrical cardboard food containers for transporting nymphs to the cages. Plants in these cages also failed to show symptoms of the virus disease. Although no positive transmission was obtained in these tests further tests appear desirable using improved techniques for handling the insect.

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Effect of Larval Diet on the Seasonal Occurrence of Diapause in the Pink Bollworm[1]

PERRY L. ADKISSON,[2] *Texas Agricultural Experiment Station, College Station*

ABSTRACT

The pink bollworm, *Pectinophora gossypiella* (Saunders), was reared in an open insectary and in the laboratory on three synthetic diets. Diets utilized were a 5% cottonseed meal diet, a 1% cottonseed meal diet and a wheat germ diet. The 5% meal diet had the highest oil content and the wheat germ diet the lowest. Cultures were placed in the insectary and laboratory locations at weekly intervals from late June to early November. Results indicated that diet had little effect on the occurrence of diapause until other inductive stimuli came into play. The highest incidence of diapause then occurred on the diets having the greatest oil contents. When the period between sunrise and sunset decreased to approximately 12 hours (mid-September) or less, the incidence of diapause increased abruptly in both the insectary and the laboratory. Temperature appeared to have an additive effect to the photoperiodic stimulus; however, temperature alone may be sufficient stimulus to induce diapause in the pink bollworm.

The pink bollworm, *Pectinophora gossypiella* (Saunders), in temperate zones of the world may undergo a facultative diapause in the last larval instar. Diapause usually occurs during the latter part of the growing season when host plants are maturing and day lengths and temperature are decreasing. Diapause larvae may remain in the last larval instar for periods ranging from a few weeks to 31 months (Ballou 1919). Nondiapause larvae held at optimum temperature for development will usually attain the pupal stage within 20 days after hatching (Adkisson et al. 1960a, b).

Temperature, quality of the larval diet, and moisture content of the plant have been associated with the initiation of diapause in field populations of the pink bollworm (Willcocks 1916, Squire 1940, Fife 1949). However, Pearson (1958), in a study of assembled data collected from pink bollworm-infested regions of the world, theorized that photoperiod was a factor influencing diapause since the wholesale occurrence of this phenomenon is confined to short day regions. He emphasized that day lengths alone cannot completely explain diapause in the insect and that other factors such as temperature and larval nutrition also have important functions. Lukefahr (1961) using infested cotton plants grown in bio-climatic cabinets was the first to demonstrate that the pink bollworm could be induced to diapause under conditions of short days and long nights. Bull & Adkisson (1960) also reported a similar finding with larvae reared on synthetic diets. The latter authors also found that temperature and larval diet had an influence on the induction of diapause in this species. Thus, it appears that the induction of diapause in the pink bollworm may be influenced by a number of factors among which are temperature, larval nutrition and photoperiod.

PROCEDURE.—Pink bollworm larvae maintained on three synthetic diets were placed at weekly intervals in an open insectary and in the laboratory in order to study the seasonal occurrence of diapause. The first cultures were installed in each location on June 28, 1960, and the last on on the following November 2.

Diets used were a cottonseed meal diet and a wheat germ diet (Adkisson et al. 1960b, Bull & Adkisson 1960). The cottonseed meal diet was modified by solvent extraction of the raw crushed meal with n-hexane to less than 1.0% oil content by weight. Two cottonseed meal diets having different oil contents were used. The diets, hereafter designated in this paper as the 5% and 1% meal diets, had the oil content adjusted by the addition of a refined cottonseed oil (Wesson oil) in the amount of 5% or 1% (w/w) of the total diet, respectively.

Insects were reared in individual vials according to the methods outlined by Adkisson et al. (1960a, b). The vials were held in wire test-tube-type racks. Each week 144 newly hatched larvae were placed in vials containing each diet. Half of the insects on each diet were placed in the insectary and the other half were held in the laboratory on open shelves.

The criterion used for determination of diapause was the failure of any larva to pupate after a given period. Percentage of diapause was calculated from the total number of insects recovered from each diet by the time of the final record. This determination was later supplemented by a chemical analysis of the diapause larvae for ether-extractable fat.

Insects reared in the laboratory were held for 35 days before the first records were taken. This was sufficient time for nondiapause insects to attain the pupal stage. Any larvae in the cultures at this examination were held an additional 30 days before the final records were taken. Only the final records are reported in this paper, and thus, include only those larvae that had not attained the pupal stage by the end of the 65-day period.

Insectary-reared insects were held for an arbitrary period that was determined by the physiological stage of development. It was decided at the beginning of the experiment that the insects would be held in the insectary at least 35 days or until the appearance of the first pupa, whichever was the longest period. Using this criterion all the cultures placed in the insectary prior to September 28 were held there for only 35 days. The September 28 cultures were maintained in the insectary for 36 days, the October 4 for 38 days, the October 11 for 42 days, the

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[2] Associate Professor, Department of Entomology in cooperation with Entomology Research Division, U. S. Department of Agriculture.

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October 18 for 45 days, the October 26 for 57 days and the November 2 for 63 days. These were the periods of time required for some pupation to occur in the respective cultures. After the insectary period was completed, the pupae and adults were removed and the larvae held in the warm environs of the laboratory for an additional 30 days before the final records were taken. Larvae which had not pupated by this time were considered to be in diapause.

All of the insectary cultures at any given date were transported to the laboratory at the same time. During the latter stages of the experiment when the temperatures were cooler than optimum for growth it was found that the insects were not able to develop as fast on the wheat germ diet as on the two cottonseed meal diets. Because of this, some of the individuals reared on the wheat germ diet during late October and November were in the early instars when transported to the laboratory, whereas, all the insects on the cottonseed meal diets were fourth-instar larvae or pupae. It is probable that this had some influence on the results reported for the insects reared on the wheat germ diet.

The insectary-reared insects were exposed to all seasonal environmental influences except rainfall and direct sunlight. The laboratory-reared insects were held on open shelves at $80° \pm 5°$ F. They were subjected to seasonal changes in day length but were not subjected to any extreme changes in temperature.

The fat content of the larvae was determined by ether extraction in a micro-soxhlet apparatus. The oil analysis of the various ingredients of the diet was made by the Cottonseed Products Research Laboratory of the Texas Engineering Experiment Station in accordance with standard analytical method Aa-5-38 of the American Oil Chemists Society.

Temperature records were obtained from a weather station located within a few hundred yards of the insectary. The readings are for the maximum and minimum daily temperatures with the mean being the average of these two extremes. Temperatures plotted in this paper are the averages of readings taken over periods of 4 days. The records for the hours between sunrise and sunset for College Station, Texas are from a publication by Lewis & Richmond (1957).

RESULTS.—The percentages of diapause larvae reared in the different diets in each location is illustrated in figure 1. A relatively small percentage of larvae entered diapause prior to mid-September. Beginning with the cultures of September 10, the percentage of diapause larvae in the test populations increased rapidly as the season progressed.

The influence of diet is indicated by the differences in the percentages of diapause larvae occurring among the insects reared on the various diets. The influence of diet did not become apparent in either location until September. This indicates that factors other than the larval diet are the primary stimuli for the induction of diapause.

In the insectary location, the greatest percentage of diapause occurred among larvae reared on the 5% meal diet. This was the only diet in which all larvae were able to attain diapause as indicated by the percentages obtained from cultures installed after October 4. The larvae reared on the 1% meal diet responded in much the same manner as those produced on the 5% meal. However, at every record taken after the general occurrence of diapause, there was a smaller percentage of diapause larvae on the 1% meal diet than on the 5%. The incidence of diapause among larvae reared on the cultures of any given date was always considerably less on the wheat germ diet than on the two meal diets.

In laboratory cultures, the incidence of diapause among

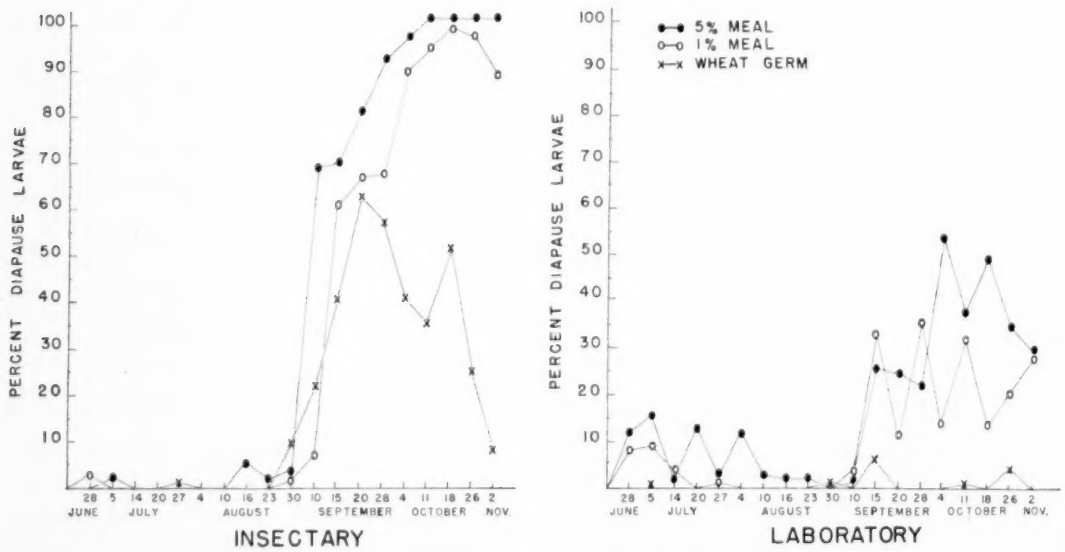

FIG. 1.—Percentage of diapause larvae that occurred in the various diets during the season. Dates shown indicate the day on which cultures of 1-day-old larvae were placed in each location. Refer to text for length of time cultures were held in each location.

Table 1.—Oil analyses of the various dietary ingredients.

INGREDIENT	GRAMS PER 100 G. DIET	OIL (%)	GRAMS OF OIL
	Wheat Germ		
Wheat germ meal	3.00	9.46	0.284
Casein	3.50	0.02	0.001
Sucrose[a]	3.50		
Salts, Wesson[a]	1.00		
Vitamins and Choline[a]	1.00 ml		
Sodium Alginate	0.50	0.04	>0.001
Agar	2.50	0.02	0.005
Water	85.00 ml		
Total	100.00	0.29	0.285
	Cottonseed Meal		
Cottonseed meal	16.25	0.97	0.158
Sucrose[a]	2.00		
Brewers' Yeast	1.50	0.75	0.011
Alphacel	1.50	0.02	>0.001
Albumin	1.00	0.03	>0.001
Agar	1.50	0.02	>0.001
Wheat germ oil	0.50	99.01	0.495
Wesson Oil	1.00 (or 5.00)	99.00	0.990
Water	75.00 ml		
Total 1% Meal	100.25	1.66	1.664
Total 5% Meal	105.25	5.34	5.624

[a] No analyses were made for these ingredients.

all larvae on all diets was less than for those reared in the insectary. Generally, the percentage of diapause was greatest among larvae reared on the 5% meal diets. However, in two cultures (September 15 and 28) the highest incidence of diapause occurred among larvae reared on the 1% meal diets. The incidence of diapause, though relatively small, was greater among laboratory cultures on all diets than in the insectary prior to early September. About mid-September the laboratory-reared insects on the 5% and 1% meal diets exhibited a seasonal response similar to that shown by larvae reared in the insectary. Although the trend was the same, the response was not as great in the laboratory as in the insectary. This response apparently was not related to temperature.

It is interesting to note that laboratory cultures maintained on the wheat germ diet did not exhibit this seasonal response. The percentage of diapause larvae in any given laboratory wheat germ culture never exceeded 6%. Diapause larvae were found in only five of the 19 weekly wheat germ cultures maintained during the experiment. This compared with diapause larvae in 13 of the 1% and 18 of the 5% meal cultures.

Since the oil contents of the diets appear to be associated with the relative percentages of diapause larvae occurring on the various media, chemical analyses for oil content of the ingredients of each were made. These analyses are presented in table 1. The 5% meal diet contained the most oil and the wheat germ the least.

When the percentages of diapause larvae occurring in each location on all diets is summed, averaged and plotted against prevailing day lengths and temperatures, the relationship of these factors to the seasonal incidence of diapause can be illustrated better, figure 2. The abrupt increase in the incidence of diapause in mid-September cannot be explained on the basis of diet or temperature alone. That temperature is not the only factor involved is suggested by the simultaneous increases in the percentages of diapause larvae in each location. This indicates that another stimulus was also operating. The diapause response exhibited by the larvae during September can be reasonably explained on the basis of decreasing photoperiods (or lengthening nights).

The increase in percentages of diapause larvae in both locations became pronounced when the period between sunrise and sunset reached approximately 12 hours. As the length of day became shorter, the incidence of diapause among the larvae increased in both the laboratory and insectary. If 1 hour can be added to the period between sunrise and sunset to allow for any effective light of dawn and dusk, it appears that the critical photoperiod for the induction of diapause in the pink bollworm is approximately 13 hours.

If it can be assumed that the photoperiod exerted a nearly equal effect on the insectary and laboratory cultures, it is obvious that factors other than photoperiod were also influencing the induction of diapause. As indicated in figure 2, the incidence of diapause in all cultures installed after early September was higher in the insectary than in the laboratory. The average overall percentage of larvae in diapause in the laboratory for any given date never exceeded 25%. The percentages of diapause larvae in the insectary during this same period was considerably greater.

Since the laboratory cultures were held at temperatures within the range for optimum growth, the differences in percentages of larvae to attain diapause between the insectary and the laboratory indicate the effect of temperature on the induction of this phenomenon. The results suggest that temperature plays an important role in determining the ultimate percentage of pink bollworm larvae to attain diapause. Photoperiod alone did appear to furnish sufficient stimulus to induce diapause in insects reared on the wheat germ diet.

The effect of temperature is shown by the records of larvae reared in the insectary on the wheat germ diet during the cooler periods of the study. The maximum percentage of diapause larvae on the wheat germ diet occurred in the September 20 culture when 62.5% of the insects attained this condition, figure 1. The percentage decreased from this peak as the season progressed. Since the larval development on the wheat germ diets during the cooler periods proceeded at a slower rate than in those insects on the cottonseed diets, many of the larvae reared on wheat germ were transferred to the laboratory before they had attained the last instar. Therefore, many of these wheat-germ-diet-reared larvae which had not attained the diapause condition at time of transfer completed development to the adult stage before final records were taken.

In addition, the survival rate of the larvae reared in the insectary on the wheat germ diets during the latter stages of this study was considerably less than that on the cottonseed meal diets. Survival data of the larvae are presented in table 2. The average survival of pink bollworms, when computed as a percentage of the original number of larvae placed on the media, was greater prior to August 30 on the wheat germ and 1% meal diets than on the 5% meal diet regardless of location. There was little difference in survival before August 30 between insects reared in the insectary and the laboratory. After August 30, larval

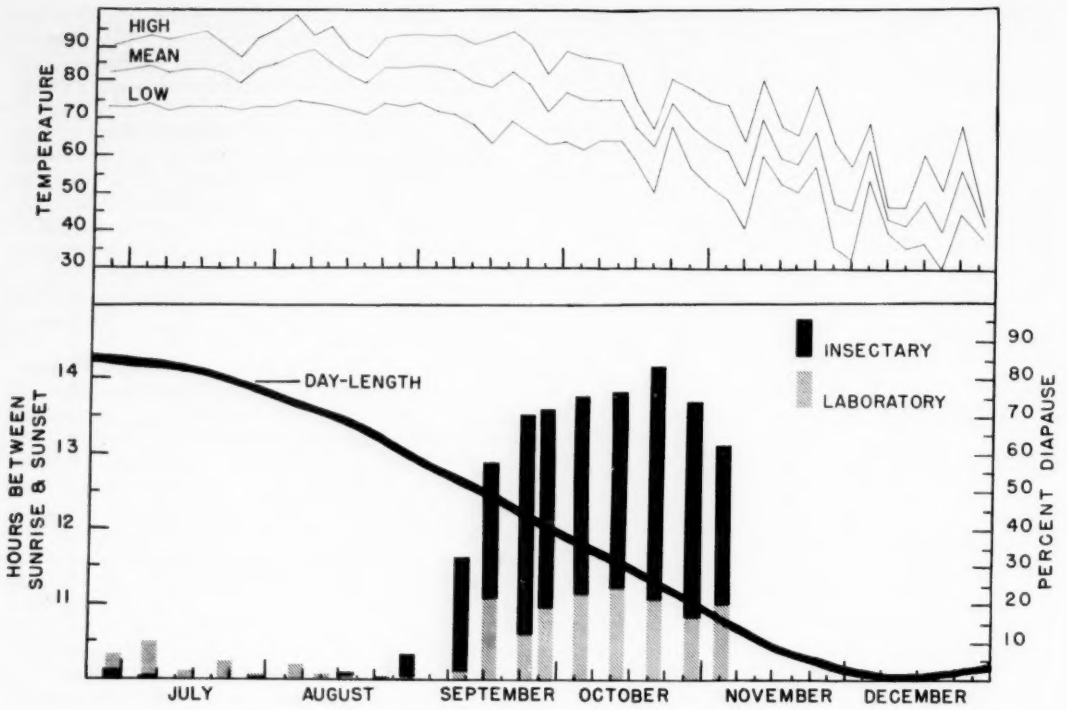

FIG. 2.—The average seasonal occurrence of diapause for all diets in each location as related to prevailing day lengths and photoperiods. Dates indicate the days on which cultures of 1-day-old larvae were placed in each location.

survival was somewhat less in the insectary than in the laboratory. The difference in survival on the 1% and 5% meal diets probably was not significant. Survival on the wheat germ diet was considerably lower in the insectary than in the laboratory after this date. As the temperature decreased mortality of the larvae on the insectary wheat germ diets increased. Mortality in the larvae reared on the wheat germ diet occurred in the early instars before diapause was attained.

Fat analyses, based on dry weight, of larvae classed as diapause are presented in table 3. The 42% to 46% fat content obtained is comparable to the percentages reported by Squire (1940) who demonstrated that diapause larvae have higher fat contents than nondiapause larvae.

DISCUSSION.—It is apparent that larval diet has an important function in determining the ultimate percentage of the pink bollworm population to attain diapause. That the larval diet, per se, is not able to induce diapause is indicated by the small percentages of diapause larvae occurring before mid-September and by the fact that very few larvae reared in the laboratory on the wheat germ diet entered diapause. The results indicate that oil content of the larval diet may act as a prediposing factor for the occurrence of diapause in the pink bollworm because as soon as diapause-inducing stimuli such as the appropriate photoperiods and temperatures come into play, the incidence of diapause was always greater on the diets having the highest oil contents.

Also, it appears that appropriate photoperiods cannot provide sufficient stimuli to induce diapause unless the larval diet is of appropriate quality. This is shown by the reaction of the pink bollworms reared in the laboratory on the wheat germ diet which were apparently able to avert any diapause-inducing stimuli attributable to photoperiod. The response of the individuals reared on the wheat germ diet may be comparable with that of field-collected larvae from squares and blooms in which a low incidence of diapause has been commonly found.

Whether the response of the larvae reared on the wheat germ diet is due to the absence of some factor (or to inadequate quantities of the same) or a diapause inhibitory substance is not known. Evidence accumulated in this study suggests that the oil content of the larval diet has an important function in the control of diapause. This substantiates previous work by Vanderzant & Reiser (1956) which indicated that the incidence of diapause in pink bollworms reared on a casein diet under comparable rearing regimes could be increased by increasing the fat content of the diet.

The pink bollworm may be considered a short-day insect since diapause is induced when periods between sunrise and sunset become less than approximately 12 hours. Photoperiod furnishes the only adequate explanation for the sudden onset of diapause in Central Texas in the early fall when temperatures are usually adequate for development at a normal rate. This is substantiated by the simultaneous increase in the percentage of diapause larvae occurring in the insectary and laboratory popula-

tions. If temperature were the only stimulus involved, there should have been no increase in diapause in the laboratory population. The sudden onset of diapause in the insectary population reared on the cottonseed meal diets closely follows that reported by Fife et al. (1957) for pink bollworm larvae collected from cotton fields of Central Texas. The average percentage of diapause calculated from his data for 1955 and 1956 shows that less than 10% of the larvae entered diapause prior to September, that approximately 30% were in diapause by mid-September, 70% by the last of September and that practically all the larvae collected in October and November had attained diapause.

The response of the pink bollworm to decreasing photoperiods suggests a possible method for drastically reducing or even eliminating overwintering pink bollworm populations. This might be done by chemically defoliating or desiccating host plants before photoperiods are short enough to induce diapause. That this practice may have possibilities is suggested by the work of Adkisson et al. (1958), which indicated that chemical termination of cotton plants produced an unsatisfactory host for the buildup of late fall population of the pink bollworm. If subsequent research should prove the practicality of this concept, it may be possible to forecast accurately, on the basis of day length, the onset of diapause in the pink bollworm population of any given region.

The importance of temperature is illustrated by the difference in percentages of diapause occurring in all diets at both locations, even after the photoperiodic stimulus had come into play. These differences can reasonably be attributed to the cooler temperatures prevailing in the insectary. The results indicate that temperature may have an additive effect to the response elicited by photoperiod.

In summary, the results of this experiment indicate that quality of the larval diet, photoperiod and temperature all may influence the ultimate percentage of pink bollworm larvae that attain dapause. The influence of diet can be averted when the periods between sunrise and sunset are greater than approximately 12 hours provided the temperature is optimum for development. Conversely, the diapause-producing influence of photoperiod can be averted by manipulation of the larval diet as demonstrated by the response of insects reared in the laboratory on the wheat germ diet. Evidence of interactions among these three stimuli was demonstrated. Further research is needed to analyze these interactions better.

Table 2.—Survival of pink bollworm on each diet in both locations.

Date Culture Installed	Days in Insectary	Media: 5% Meal Insects Recovered Number[a,b]	5% Meal (%)	1% Meal Insects Recovered Number[a,b]	1% Meal (%)	Wheat Germ Insects Recovered Number	Wheat Germ (%)
		Insectary					
Before 8/30	35	395	61.0	465	71.8	517	79.8
8/30	35	47	65.3	51	70.8	61	84.7
9/10	35	35	48.6	15	20.8	49	68.1
9/15	35	49	68.1	51	70.8	49	68.1
9/20	35	37	51.4	56	77.8	40	55.0
9/28	36	59	81.9	63	87.5	53	73.6
10/4	38	53	73.6	57	79.2	54	75.0
10/11	42	55	76.4	49	68.1	37	51.4
10/18	45	52	72.2	60	83.0	29	40.3
10/26	57	48	66.7	53	73.6	16	22.2
11/2	63	45	62.5	55	76.4	6	8.3
After 8/30 Total		480		510		394	
Average			66.7		70.8		54.7
		Laboratory					
Before 8/30		370	57.1	517	79.8	524	80.9
8/30		45	62.5	50	69.5	52	72.2
9/10		51	70.8	23	31.9	65	90.3
9/15		57	79.2	64	88.9	16	22.2
9/20		53	73.6	53	73.6	66	91.7
9/28		59	81.9	50	69.5	60	83.3
10/4		46	63.9	62	86.1	66	91.7
10/11		29	40.3	65	90.2	65	90.2
10/18		63	87.5	67	93.1	57	79.2
10/26		55	76.4	64	88.9	69	95.8
11/2		61	84.7	69	95.8	63	87.5
After 8/30 Total		519		567		579	
Average			72.1		78.8		80.4

[a] 72 larvae were originally installed on each media for each date at each location. Survival calculated as the percentage of insects recovered at the end of the test.

[b] Represents total insects recovered and average survival for the nine cultures prior to August 30 when there was slight variation between cultures and dates as pertaining to survival.

Table 3.—Average percentage fat content of diapause pink bollworm larvae reared on three diets in two locations.

Source	Number Analyses	Total Insects Used	Per Cent Fat Dry Wt. Basis
5% meal, Insectary	9	135	45.7
5% meal, Laboratory	11	150	42.6
1% meal, Insectary	8	120	43.9
5% meal, Laboratory	11	130	42.2
Wheat germ, Insectary	7	96	42.1
Wheat germ, Laboratory	0	0	—

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Effectiveness of *Bacillus thuringiensis* and Several Chemical Insecticides for Control of Budworms and Hornworms on Tobacco[1]

C. S. CREIGHTON,[2] W. S. KINARD,[3] and NORMAN ALLEN,[2]
Entomology Research Division, Agric. Res. Serv., U.S.D.A.

ABSTRACT

In a field experiment conducted at Florence, S. C., in 1960, a commercial *Bacillus thuringiensis* Berliner spore material containing 93×10^9 spores per gram gave good control of the tobacco hornworm (*Protoparce sexta* (Johannson)) and the tobacco budworm (*Heliothis virescens* (F.)) on tobacco. Hand applications of the spore material, either undiluted or in a cornmeal bait, to the terminal buds gave as good or better control than sprays containing Thiodan® (6,7,8,9,10,10-hexachloro-1,5,5*a*,6,9,9*a*-hexahydro-6,9,-methano-2,4,3-benzodioxathiepin-3-oxide), endrin, or Shell SD-4402 (1,3,4,5,6,7,8,8-octachloro-3*a*,4,7,7*a*-tetrahydro-4,7-methanophthalan), but caused injury to tender foliage. A bacillus suspension spray was less phytotoxic and equal to the chemical insecticides in protecting the plants from the caterpillars. Combinations of the bacillus and either SD-4402 or Thiodan were usually no better than the bacillus alone.

Two of the major insect pests of flue-cured tobacco in South Carolina are the tobacco hornworm (*Protoparce sexta* (Johannson)) and the tobacco budworm (*Heliothis virescens* (F.)). The future for the utilization of microbial organisms to control these insects on tobacco looks very promising. Rabb et al. (1957) found that the spore-forming bacterium *Bacillus thuringiensis* Berliner was pathogenic to the tobacco hornworm and the tomato hornworm (*Protoparce quinquemaculata* (Haworth)). Guthrie et al. (1959) reported that *B. thuringiensis* was equal to endrin in preventing hornworm damage although not so effective initially in reducing larval populations.

In 1957, 1958, and 1959 the authors found that high dosages of two commercial formulations of *B. thuringiensis* applied as sprays to the tobacco foliage were effective against hornworms but ineffective against budworms. During 1959, however, a bacillus formulation was effective against budworms when mixed with different amounts of cornmeal and applied by hand as a bait. Cornmeal was used in the baits because this material has been mixed with insecticides and used as a bait for budworm control for many years.

This paper reports results of an experiment conducted in 1960 to determine the effectiveness of seasonal applications of one commercial formulation of *B. thuringiensis* with and without several chemical insecticides against the tobacco hornworm and the tobacco budworm. The chemical insecticides were also tested alone in order to compare their effectiveness with that of the bacillus.

MATERIALS AND METHODS.—The insecticides and the formulation of *B. thuringiensis* tested, with the rate of actual materials applied per acre, are shown in table 1.

The *B. thuringiensis* spore material was a wettable-powder formulation with a count of 93 billion spores per gram, designated as Batch 9A, and supplied by Rohm & Haas Company.

All chemical insecticides were tested as emulsion sprays. *B. thuringiensis* was applied as sprays with or without Thiodan® (6,7,8,9,10,10-hexachloro-1,5,5*a*,6,9,9*a*-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide) or Shell SD-4402 (1,3,4,5,6,7,8,8-octachloro-3*a*,4,7,7*a*-tetrahydro-4,7-methanophthalan) or as baits with or without an SD-4402 spray applied separately. The sprays were applied at the rate of 75 gallons per acre with a mule-drawn traction sprayer equipped with an adjustable spray boom and six nozzles per row.

The bacillus-cornmeal baits were applied by hand to the buds of the tobacco plants at the rate of 12½ pounds per acre and the undiluted spore material was applied in the same manner at 10 pounds per acre. In applying the combination *B. thuringiensis* bait-SD-4402 treatments, the plants were sprayed with the insecticide previous to the application of the baits. All treatments were applied on June 7, 28; July 13, and 27.

The experiment was conducted on a farm at the Clemson College Pee Dee Experiment Station in a randomized-block design with four replications. Each plot consisted of four rows of plants and was 1/80-acre in size. The plants were set on May 3 and the necessary replants on May 11.

Two methods were used to determine the effectiveness of the treatments. The first consisted of counting the budworm and hornworm larval populations throughout the growing season on 25 plants on the two middle rows of each plot on each of the following dates: June 9, 13, 20, 27; July 5, 12, 18, 25; and August 1. The second method consisted of estimating the hornworm and budworm injury by a visual technique developed by F. R. Lawson[4] (unpublished). A total of 20 leaves from each of 18 tobacco plants per plot were removed and examined during the growing season as follows: Three leaves each on July 7, 14, 20; five leaves on July 28; and six leaves on August 8. The amount of foliage eaten from each leaf by either budworms or hornworms was recorded separately in units of 1, 2, 3, or 4 quarters. Injury of less than ⅛ of the leaf was not considered. Injury amounting to more than ⅛ of the

[1] Accepted for publication May 8, 1961.
[2] Entomology Research Division, Agric. Res. Serv., U.S.D.A.
[3] South Carolina Agricultural Experiment Station.
[4] Entomology Research Division, Agric. Res. Serv., U.S.D.A., at Oxford, N. C.

Table 1.—Relative efficiency of *B. thuringiensis* and insecticides against budworms and hornworms on tobacco.[a]

Treatments	Pounds/Acre	Number of		Per Cent of Leaves Eaten[b]	
		Hornworms Present[c]	Budworms Present[c]	Hornworms	Budworms
Baits (Ratio of bacillus to cornmeal)					
Without SD 4402					
1:0	10	1 a	4 abc	0.7 a	0.4 a
1:3	3.1	2 ab	3 a	.8 a	.6 ab
1:9	1.25	3 abc	3 a	.9 a	.5 ab
1:49	0.25	7 de	3 ab	1.9 bcd	.9 abc
1:99	0.125	10 e	4 abc	1.9 bcd	1.6 abcd
With SD 4402 at 0.156 pound per acre					
1:3	3.1	2 ab	4 abc	1.3 ab	0.7 ab
1:9	1.25	2 ab	4 abc	0.8 a	.7 ab
1:49	0.25	4 abcd	6 abcd	1.0 ab	.7 ab
1:99	0.125	5 cd	4 ab	0.9 a	1.0 abc
Sprays					
B. thuringiensis	4.0	5 cd	12 fg	2.5 cde	1.9 bcd
Plus Thiodan	4.0+0.5	4 abcd	10 ef	2.5 cde	1.8 abcd
Plus SD 4402	4.0+0.156	5 cd	8 cde	1.9 bcd	2.3 cde
Thiodan	0.5	5 cd	9 def	3.4 e	3.4 ef
SD 4402	0.156	6 cd	7 bcde	1.6 abc	1.9 bcd
Endrin	0.2	3 abc	12 fg	2.7 de	2.7 de
Untreated check	—	23 f	15 g	7.5 f	4.35 f

[a] Values followed by the same letter are not significantly different at the 5% level.
[b] Calculated on the basis of 100 plants per treatment.
[c] Average number of worms per 100 plants per treatment for 9 weekly observations.

leaf, but less than $\frac{1}{4}$, was recorded as $\frac{1}{4}$, and so forth. By summing the recorded fractions an estimate of the number of leaves destroyed was obtained and a per cent computed.

RESULTS.—All concentrations of the bacillus-cornmeal baits gave good control of budworms (table 1). The bacillus baits used alone gave better control than either endrin or Thiodan alone. No significant differences were shown between any of the bacillus baits, with or without the addition of SD-4402, as indicated by budworm populations or leaves eaten by budworms. The bacillus spray did not reduce the budworm population below endrin treatment or the untreated check; however, it was just as effective as the bacillus baits in stopping budworm feeding. No significant differences in the number of leaves eaten were shown with endrin, SD-4402, or the bacillus spray; however the SD-4402 was superior in reducing the population. Thiodan or SD-4402 did not increase the effectiveness of the bacillus spray in reducing budworm feeding.

The *B. thuringiensis* spray was equal in effectiveness to the endrin spray in reducing the hornworm population and the amount of hornworm injury. No significant differences were shown between SD-4402, Thiodan, or endrin in reduction of hornworm population; however, SD-4402 was superior in reducing hornworn feeding. The addition of either SD-4402 or Thiodan to the bacillus spray did not increase its effectiveness.

Hornworm populations were slightly higher on the plants treated with the bacillus-cornmeal baits at ratios of 1:49 or 1:99 than on the endrin-treated plants, but the control of hornworms as measured by the amount of hornworm feeding on these plants was as good as on the endrin-treated plants.

In reduction of the amount of hornworm feeding, the bacillus-cornmeal baits at ratios of 1:0, 1:3, and 1:9 were significantly more effective than endrin or Thiodan used alone, the bacillus spray used alone, or the combination bacillus-chemical sprays.

Plant injury from the bacillus treatments was observed a few days after the first application. The most pronounced injury occurred to the tender leaves near the top of the plants that received periodic hand applications of the bacillus spore material, either undiluted or in cornmeal baits. The first sign of injury was characterized by bleached areas in the leaf generally confined to a line parallel to the leaf margin. In many instances the injury resulted in distorted leaves and, where the injury was severe, necrotic spots were formed and those later fell out of the leaf.

In order to determine the extent of this injury, 25 plants of each plot were examined on July 7, 9 days after the second application. The percentages of tobacco plants showing one or more leaves injured by the bacillus-cornmeal bait and the bacillus spray are given in the following tabulation:

Formulations Baits (Ratios)	*Pounds of Bacillus/Acre*	*Plants with One or More Injured Leaves (%)*
1:0	10.0	95
1:3	3.1	83
1:9	1.25	68
1:49	0.25	17
1:99	0.125	8
Sprays	4.0	5

These data indicate that the sprays caused less plant injury than the baits and that the amount of injury caused by the baits was inversely related to the bacillus content.

Tests conducted in previous years with other bacillus

formulations resulted in no noticeable phytotoxicity to the tobacco foliage. The phytotoxicity resulting from some of the bacillus treatments in 1960 was serious from an appearance standpoint.

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Experiments on the Control of Poultry Lice[1]

ROBERT A. HOFFMAN, *Entomology Research Division, Agric. Res. Serv., U.S.D.A.*

ABSTRACT

Control of poultry lice by spraying the bird, dusting the litter, and feeding has been attempted with several new insecticides. Sprays of dimethoate, and Hercules 426 (5-dichloromethyl-1,2,3,4,7,7-hexachlorobicyclo[2.2.1]hept-5-ene) gave mortalities similar to the malathion standard at 0.05%. Dibrom® (1,2-dibromo-2,2-dichloroethyl dimethyl phosphate) was outstanding in providing 100% mortality at 0.025%. Sevin® (1-napthyl *N*-methylcarbamate) and Co-Ral® (*O*-(3 chloro-4-methylumbelliferone) *O,O*-diethyl phosphorothioate) in 5% dusts were effective at one-half pound to 40 square feet under controlled conditions but were erratic under variable farm conditions. Orally administered toxicants were generally ineffective.

The poultry of North America may become infested with one or more of seven species of lice (Fairchild & Dahm 1955). In the mid-South the chicken body louse (*Menacathus stramineus* (Nitzsch)), and the shaft louse (*Menopon gallinae* (L.)) are particularly common. The wing louse (*Lipeurus caponis* (L.)), and the fluff louse (*Goniocotes gallinae* (De Geer)), are also found, and may be abundant on an occasional flock.

Several workers have reported experiments with some of the newer insecticides for control of poultry lice (Edgar et al. 1949, Smith 1952, Fairchild & Dahm 1955, Rodriguez & Riehl 1958, Linkfield & Reid 1958, and Kraemer 1959). The present paper reports results of studies on control of poultry lice conducted in the vicinity of Stoneville, Miss., in 1959 to evaluate various toxicants, formulations, and application methods. The studies are in part a continuation of earlier work on poultry parasites (Hoffman 1960).

METHODS AND MATERIALS.—Four separate experiments were conducted. These included direct spraying of individual birds in screening tests, dusting of litter in range houses under identical conditions at the experiment station, dusting of litter in farm poultry houses under variable conditions, and oral feeding of the birds with insecticides to determine their chemotherapeutic value against nonblood-feeding lice.

Delnav, dimethoate, ronnel, and Sevin are promising materials for which data have been previously presented but their use was repeated primarily because of a technique change in the direct-spray testing procedure. Malathion was included as a standard.

LOUSE POPULATION DATA.—The chicken body louse and the shaft louse were the common species found on all birds in the tests. The wing louse was observed on a few birds but was not included in the data. The northern fowl mite (*Ornithonyssus sylviarum* (Canestrini & Fanzago)) was also present on some of the birds.

In experiments I and IV, population densities were established on individual birds but in experiments II and III an average count from 10 birds caught at random was the basis of control data. Ordinarily two pretreatment counts were made, the second one immediately before treatment. The count assigned an individual bird was the total number of lice observed in 10 openings of the feathers: two each at vent, breast, back, neck, and wings. In general, the population was greatest near the vent; but in severe infestations lice were found on all parts of the body.

Birds in individual wire cages (experiments I and IV) were ordinarily subject to reinfestation from untreated birds in adjoining cages, whereas birds on litter were either confined in isolated range houses (experiment II), or allowed the freedom of both house and farmyard (experiment III).

Insecticides tested and the chemical identification of proprietary materials are as follows:

Co-Ral® (*O*-(3-chloro-4-methylumbelliferone) *O,O*-diethyl phosphorothioate)
Deet
Delnav® (2,3-*p*-dioxanedithiol *S,S*-bis(*O,O*-diethylphosphorodithioate) 68–70% and related materials)
Dibrom® (1,2-disbromo-2,2-dichloroethyl dimethyl phosphate)
Dimethoate
Hercules 426 (5-dichloromethyl-1,2,3,4,7,7-hexachlorobicyclo [2.2.1]hept-5-ene)
Malathion
Ronnel
Ruelene® (*O*-4-*tert*-butyl-2-chlorophenyl *O*-methyl methylphosphoramidate)
Sevin® (1-naphthyl *N*-methylcarbamate)
Toxaphene

PROCEDURES, RESULTS, AND DISCUSSION.—*Experiment I.*—Chickens were principally White Rock and White Leghorns and were about equally divided between roosters and hens. They were housed individually in multiple-unit wire cages suspended about 3 feet above the floor.

Emulsion or wettable-powder sprays at concentrations of 0.0125 to 1.0% were applied to individual birds at the rate of 40 ml. per bird. A coarse, cone-shaped spray was delivered through a Schrader[2] spray gun described by

[1] In cooperation with the Delta Branch of the Mississippi Agricultural Experiment Station. Accepted for publication May 9, 1961.

[2] Mention of this proprietary product does not necessarily imply its endorsement by the U. S. Department of Agriculture.

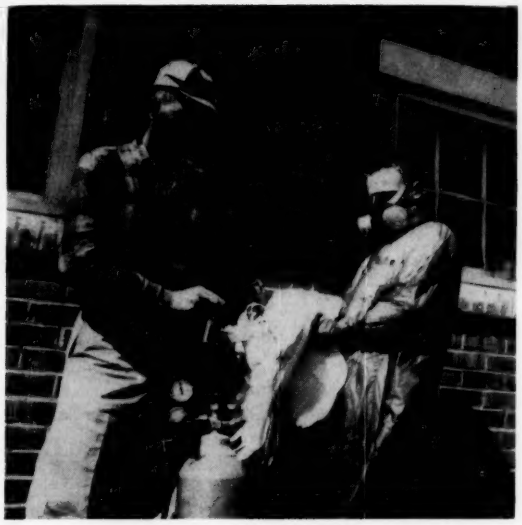

FIG. 1.—Direct spraying of individual birds.

Kilpatrick & Johnson (1956) at 20 p.s.i. under pressure supplied by a carbon dioxide cylinder. This technique provided a volume and droplet size that compared more closely with commercial recommendations than the 25-ml. volume and fine droplet size reported in earlier work (Hoffman 1960). The new technique markedly improved wetting of the bird. One man held the bird by the lower legs and slowly rotated it while a second man operated the sprayer (fig. 1). On any one spray date all treatments were applied in duplicate.

The increase in volume and larger droplet size of the sprays and the method of application were believed

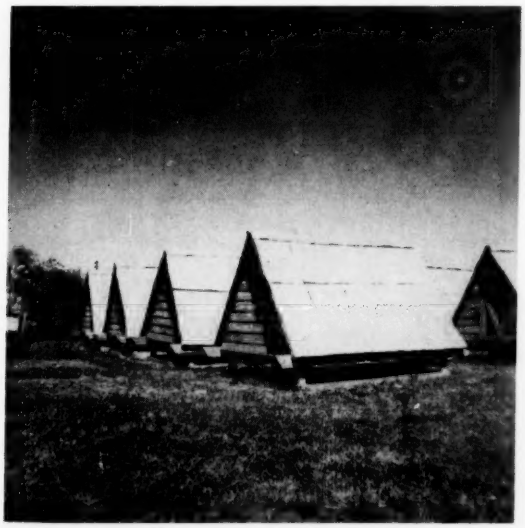

FIG. 2.—Range houses at Delta Station.

Table 1.—Control of poultry lice by direct application to the birds of various insecticides. Experiment I.

INSECTICIDE (%)	PER CENT CONTROL AFTER INDICATED DAYS					
	1	3	5	10	15	20
Delnav						
0.05	21	42	64	77	73	80
.1	38	77	92	95	99	99
.25	85	100	100	100	100	100
Ronnel						
0.1	91	100	100	100	100	100
.25	100	100	100	100	100	100
.5	100	100	100	100	100	100
Toxaphene						
0.1	85	100	100	100	100	100
.25	100	100	100	100	100	100
.5	100	100	100	100	100	100
1.0	91	100	100	100	100	100
Malathion						
0.025	85	91	96	90	88	82
.05	97	100	100	100	100	100
.1	100	100	100	100	100	100
.25	100	100	100	100	100	100
Sevin						
0.025	63	90	94	100	100	99
.05	91	96	100	97	85	97
.1	90	90	100	100	100	100
.25	94	100	100	100	100	98
Dimethoate						
0.05	89	96	100	100	100	100
.1	84	99	100	100	100	100
Dibrom						
0.0125	100	100	92	86	84	79
.025	100	100	100	100	100	100
.05	100	100	100	100	100	—
.1	100	100	100	100	100	—
Hercules 426						
0.0125	57	55	57	47	48	34
.025	85	100	100	100	94	98
.05	100	100	100	100	100	100
.1	100	100	100	100	100	100
.25	100	100	100	100	100	100

responsible for control of lice at lower concentrations of these organic insecticide sprays than was reported previously (Hoffman 1960). However, no marked change occurred in the order of relative effectiveness of the different insecticides. At least three of the materials, dimethoate, Dibrom, and Hercules 426, provided control equal to the standard malathion (table 1). Dibrom appeared outstanding; even a 0.025% emulsion of this insecticide provided complete mortality through 20 days of observation.

Experiment II.—All chickens in the range house litter tests were hens of the white breeds. The ten 40-square-foot houses were of tight construction except for a 6-inch screened opening around the base and a small screen door at one end (fig. 2). A 4-inch layer of crushed-sugarcane poultry litter covered the ground.

Dust applications were made at the rate of 0.25 to 1 pound per 40 square feet of floor from glass jars having nail holes in their metal lids. The litter surface was lightly scratched with a rake to reduce loss by wind and help the

Table 2.—Poultry lice control by five insecticide dusts applied to litter under equal conditions. Experiment II.

Insecticide (%)	Pounds/ 40 Sq. Ft.	Per Cent Reduction After Indicated Days						
		3	5	7	10	15	20	25
Sevin, 5	0.25	67	76	82	84	91	88	—
	0.5	100	100	100	100	100	100	—
Ronnel, 4.6	0.5	65	56	88	78	49	55	—
	1	97	100	100	100	100	100	—
Delnav, 10	0.25	0	64	45	62	36	67	—
	0.5	53	55	55	76	76	86	92[a]
	1	99	100	100	100	100	100	100
Co-Ral, 5	0.25	86	98	98	100	96	91	89
	0.5	99	100	96	93	100	100	—
Deet, 15	0.5	49	41	41	57	46	62	—

[a] 72% after 30 days.

dust adhere to the litter particles. Coverage was considered good when 0.5 or 1 pound of dust to 40 square feet was applied, but 0.25 pound appeared to spread rather thin.

In litter treatments the efficacy of an insecticide depends upon the fumigant properties of the toxicant and the birds' direct contact with the treated litter while dusting themselves. In these tests the fumigation effect was minimized by cross-ventilation resulting from the open structure of the houses immediately above the litter. These data are therefore considered primarily the result of contact insecticides.

Data on Sevin and ronnel confirm and extend previous results with these insecticides. Co-Ral appeared to give results that were less consistent than those obtained with Sevin, but provided good control of lice at 0.5 pound to 40 square feet. The results are summarized in table 2.

Experiment III.—In the six tests comprising experiment III, hens of both dark and light breeds were involved, but on any one farm the color was primarily one or the other. The poultry houses ranged in size from 64 to 420 square feet. All houses were of wood; most of them had little ventilation except the entrance door and small openings near the floor which were used by the birds for entering or leaving. Nests were of wood construction and usually placed about 3 feet above the floor. Litter consisted of either loose hay, sawdust, or corncob and shuck. Loose hay was used for nest bedding.

Dust treatments were applied in the same manner and at the same rate as in experiment II; however, the dust was not readily distributed through the bulky hay and corncob litters.

Only in two of the six litter tests was satisfactory control obtained with Sevin or Co-Ral under typical small-farm conditions (table 3). It appeared that management practices on cooperators' farms, especially in relation to the use and condition of litter materials, were responsible for failures with dust treatments similar to those that had been successful in experiment II.

Experiment IV.—All birds in experiment IV were Leghorn roosters. Each was housed in an individual wire cage to assure an exact feeding level adjusted to the 2.5-kilogram weight of the birds. The feed was prepared by suspending the technical insecticides in amounts ranging from 25 to 500 mg. in acetone or water and pipetting the suspension on about 10 grams of poultry pellets. The diluents were allowed to evaporate before the pellets were offered to the birds. The birds were given no other food until they had completely consumed the insecticide ration. In these tests only a single oral dose was given to each bird.

Most of the poultry accepted the treated food without

Table 3.—Control of poultry lice in farm flocks with litter treatments of Co-Ral and Sevin dusts. Experiment III.

Insecticide (%)	Pounds/ 40 Sq. Ft.	Kind and Amount of Litter Comments	Per Cent Reduction After Days			
			7	14	21	28
Co-Ral, 5	0.25	Hay litter, birds did not use; 2.3 sq. ft./bird	77	35	—	—
	0.25	Hay litter, birds did not use; low % laying. Little use of nests; 2.3 sq. ft./bird.	46	57	19	—
	0.5	Dry corn shuck on dirt floor; birds used; 2.8 sq. ft./bird.	99	100	96	100
Sevin, 5	0.5	Sawdust, birds fed inside and spent much time on litter; 3.5 sq. ft./bird.	73	99	98	99
	1	Sawdust, birds fed outside, spent little time on litter; 3.5 sq. ft./bird.	74	68	—	—
	1[a]	Sawdust, birds fed outside, spent little time on litter; 3.5 sq. ft./bird.	72	92	85	88

[a] Plus 1 gram in each nest.

Table 4.—Results of feeding insecticides to poultry. Experiment IV.

TOXICANT	RESULTS	MG./BIRD
Sevin	No control	250 and 500
Ronnel	No control	125, 250, and 500
Co-Ral	No control[a]	50, 75, 125, and 250
Dimethoate	No control[a]	50, 75, 125, and 250
Ruelene	Variable[b]	25, 50, 125, 250 and 500

[a] One bird fed 125 mg. and both fed 250 mg. died.
[b] Control highly variable among replicates except at 500-mg. level at which a 67% average reduction was obtained in 10 days.

delay and ate the entire ration within 2 hours. No symptoms of poisoning developed with Sevin, ronnel, or Ruelene; but Co-Ral and dimethoate produced positive symptoms of poisoning at doses as low as 75 mg. per bird and death occurred at 125 mg. per bird. Symptoms appeared within a few minutes after feeding, and included loss of coordination, salivation, diarrhea, and eventually, an inability to stand. Only Ruelene demonstrated any control of lice. The test results are summarized in table 4.

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Toxicity of Several Organophosphates Administered in the Diet of Broilers to House Fly Larvae in the Feces[1]

H. W. DOROUGH and B. W. ARTHUR,

Department of Zoology-Entomology, Auburn University Agricultural Experiment Station, Auburn, Alabama

ABSTRACT

Ronnel, butonate, Ruelene® (*O*-4-*tert*-butyl-2-chlorophenyl *O*-methyl methylphosphoramidate), Bayer 29493 (*O,O*-dimethyl *O*-[4-(methylthio)-*m*-tolyl] phosphorothioate), Bayer 22408 (naphthalimido-*O,O*-diethyl phosphorothioate), Bayer 34098 (*O,*(4-methylthio-*m*-tolyl) dimethyl phosphinothioate) and Bayer 37342 (*O,O*-dimethyl *O*-(3,5-dimethyl-4-methylthiophenyl) phosphorothioate) were mixed in poultry-growing mash at 50, 100, 200, 400, and 800 p.p.m. and fed to 5- to 7-week-old broilers for 7 days. Bayer 22408, Bayer 29493, Bayer 34098, and Bayer 37342 at all concentration levels in the feed resulted in 100% control of house fly (*Musca domestica* L.) larvae in the feces. Ronnel was effective at the 200, 400, and 800 p.p.m. levels, but was ineffective at the lower concentrations against house fly larvae; butonate and Ruelene were ineffective. Polymer and nonpolymer formulations of Bayer 22408 in the feed were equally effective against 1- and 3-day-old house fly larvae in poultry droppings.

Negative gains in weight were observed of birds receiving Bayer 29493 and Bayer 37342 at 200 p.p.m. or higher in the feed. Fifty per cent of the broilers died that were on feed containing 400 or 800 p.p.m. Bayer 37342.

Control of the house fly (*Musca domestica* L.) is one of the most serious problems of the poultryman in Alabama. Forty-five species of flies representing 18 families were collected from poultry droppings at Auburn, Alabama (Cunningham et al. 1955). In Hawaii, 12 species of diptera in poultry manure were reported by Illingworth (1923) and 24 species were reported by Tanada et al. (1950). The house fly was the most predominant species in Hawaii (Tanada et al. 1950) and in Alabama (Cunningham et al. 1955).

One of the conventional methods of controlling the house fly in poultry houses is by application of chlorinated hydrocarbon (Wolfenbarger & Hoffman 1944, Tanada et al. 1950) or organophosphate (Mayeux 1954, Hoffman & Monroe 1956) insecticides directly to poultry droppings. Such a method of house fly control is expensive and requires a considerable amount of time for repeated applications of insecticides.

Recently, the addition of insecticides to the feed of poultry for controlling dipterous larvae that develop in the droppings has received considerable attention. Polybor 3 (98% disodium octaborate tetrahydrate) added to the diet of laying hens was effective for control of fly larvae in droppings, but residues of boron were detectable in tissues and eggs (Goodman 1958, Burns et al. 1959, Sherwood 1959). Several organophosphates, administered as a single dose in the feed of 1-week-old chicks, caused greater than 90% mortality of 1-day-old house fly larvae in the droppings (Sherman & Ross 1959). When laying

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hens were fed an insecticide-treated diet for 29 weeks (Sherman & Ross 1960), the insecticide levels in the feed that caused greater than 90% mortality of 1-day-old house fly larvae in the feces were: Co-Ral®(*O,O*-diethyl *O*-3-chloro-4-methyl-2-oxo-2H-1-benzopyran-7-yl phosphorothioate), 89 p.p.m; Diazinon®(*O,O*-diethyl *O*-(2-isopropyl-6-methyl-4-pyrimidinyl) phosphorothioate), 154 p.p.m.; Dipterex®(dimethyl (2,2,2-trichloro-1-hydroxyethyl) phosphonate), 89–132 p.p.m.; ronnel, 176–220 p.p.m.; and Dow ET 15 (*O*-methyl 0-2,4,5-trichlorophenyl phosphoramidothioate), 89 p.p.m. These workers concluded that the use of the hen for the distribution of insecticides in droppings would eliminate many of the problems encountered in conventional methods of house fly larvae control.

The experiments reported herein include the toxicity of several organophosphates to poultry when administered in the feed, the toxicity of the residual insecticide or its metabolites in the feces to house fly larvae, and the influence of the insecticides on gains in weight of the birds.

PROCEDURE.—The insecticides included in this investigation were as follows: Bayer 22408 (naphthalimido-*O,O*-diethyl phosphorothioate), Bayer 29493 (*O,O*-dimethyl *O*-[4-(methylthio)-*m*-tolyl] phosphorothioate), Bayer 34098 (*O*-(4-methylthio-*m*-tolyl) dimethylphosphinothioate), Bayer 37342 (*O,O*-dimethyl *O*-*O*-(3,5-dimethyl-4-methylthiophenyl phosphorothioate), butonate, Ruelene® (*O*-4-*tert*-butyl-2-chlorophenol *O*-methyl methylphosphoramidate), and ronnel. The technical materials as received from the manufacturer were diluted with acetone (100 ml. acetone per gram of insecticide) for mixing with the feed (commercial growing mash). An appropriate amount of acetone solution of each insecticide was mixed with 10 pounds of feed to prepare concentrations of 50, 100, 200, 400, and 800 p.p.m. The acetone solution was mixed thoroughly by hand with about 2 pounds of feed, and this sample was mixed with the remaining 8 pounds of feed by passing through a blending machine several times.

In certain experiments with Bayer 22408, the final concentrations of insecticide in the feed were 10, 25, and 50 p.p.m.; technical Bayer 22408 and Bayer 22408 as a polymer were used in these experiments. The polymer formulation (Geary 1960) consisted of formalin and urea as the basic ingredients. The solidified polymer was ground to a powder with a mortar and pestle for incorporation into the feed. In one experiment the polymer powder was placed in a gelatin capsule and administered orally at 40 mg./kg.

White Rock broilers, 5 to 7 weeks of age, were housed in wire cages in groups of five birds per cage. Two groups of five birds were used for each insecticide concentration in the diet (50, 100, 200, 400, and 800 p.p.m.). Two replicates of five birds each were used as untreated controls for each insecticide. Insecticide-treated feed was fed the birds for 7 days at which time the birds were returned to normal feed for 7 days. The feed containers were located outside the cages to prevent contamination of the droppings with the feed. The droppings were collected daily on protecto paper placed underneath each cage. The paper was changed daily to avoid contamination of the feces eliminated during each 24-hour period with that previously voided. The droppings from each replicate of birds were placed in quart cardboard cartons for studying the toxicity of fecal metabolites to house fly larvae. The total weight of feces from each group of birds ranged from 100 to 150 grams per day. The cardboard cartons containing the feces were covered with a copper screen and stored in a room at 27° C. Fifty 3-day-old house fly larvae were introduced into the fecal sample and 2 ml. of water was added every other day to replace that lost by evaporation. The effectiveness of the compounds was determined from the percentage of adults emerging from each fecal sample. The number of adults was counted daily from the time the first emergence was noted. In the low level feeding of Bayer 22408 and Bayer 22408 as a polymer, the relative toxicities of the fecal metabolites to 1- and 3-day-old house fly larvae were determined. Abbott's formula (Abbott 1925) was used in the calculations to compensate for natural mortality of the house fly larvae. The house fly larvae were reared on C.S.M.A. (Chemical Specialties Manufacturers Association) media according to methods described in detail by Owen (1959).

The effect of treatment on weight gains for each group of birds was determined by weighing the birds at the beginning of the experiment, after 7 days on treated feed, and 7 days after the return to normal feed. The amount of feed consumed per bird per day was recorded and the dosage of insecticide in mg./kg. was calculated from these data.

RESULTS.—*Feed Consumption and Gains in Weight.*—The average amount of feed consumed per bird per day, the corresponding mg. per kg. intake of insecticide, and gains in weight are given in table 1. All birds on insecticide-treated feed consumed a normal amount except those receiving Bayer 29493 at 100 p.p.m. or more and those receiving Bayer 37342 at 200 p.p.m. or more. There was some variation in the amount of feed consumed by the birds receiving different insecticides in their diet. This was probably a result of variations in ages of the birds used in the experiment.

Birds receiving Bayer 29493 and Bayer 37342 at 200 p.p.m. or higher or Bayer 22408 at 800 p p.m. in the feed made negative weight gains (table 1). The loss of weight by birds receiving Bayer 29493 and Bayer 37342 was the result of limited feed consumption. However, these same birds when returned to normal feed made more rapid gains than the untreated birds. In other instances when the birds received insecticides as a feed additive for 1 week, gains in weight were comparable to those of untreated broilers. Birds receiving ronnel and Ruelene were weighed at the beginning of the experiment, but were not weighed after being on treated feed for 1 week; therefore, only the total gains in weight for the 2-week experimental period are reported (table 1.).

Gains in weights were not computed for birds receiving 800 p.p.m. of Bayer 37342 because 80% of the broilers died. There was a 50% mortality of birds that were fed Bayer 29493 at 400 and 800 p.p.m. in the diet. Actually the birds receiving these concentrations of Bayer 29493 consumed less insecticide than birds on the 100 or 200 p.p.m. levels, but there was virtually no mortality of birds receiving lower concentrations of Bayer 29493. There was no mortality of birds receiving Bayer 22408, Bayer 34098, butonate, ronnel or Ruelene mixed in the

feed at 50 to 800 p.p.m. Low-level feeding of technical Bayer 22408 and Bayer 22408 formulated as a polymer (10, 25, and 50 p.p.m.) had no gross adverse effect on the amount of feed consumed or the gains in weight of the broilers.

House Fly Mortality.—There was almost 100% control of house fly larvae in the feces from broilers fed Bayer 29493, Bayer 22408, Bayer 34098, and Bayer 37342 in the diet at all concentration levels; however, when the birds were returned to normal feed, even for 1 or 2 days, a marked decrease in degree of control resulted (table 2). Ronnel was excreted in the feces in sufficient quantity to effectively control house fly larvae at concentrations of 200, 400, and 800 p.p.m.; it was somewhat less effective at the 50 and 100 p.p.m. levels. Ruelene and butonate were ineffective against house fly larvae at all dosages tested (table 2). Mortality of house fly larvae in the feces from untreated broilers was usually 10% to 15%.

Bayer 22408 was selected for further study, since it was effective against house fly larvae at the 50 p.p.m. level (table 2); the broilers made good gains in weight (table 1),

Table 1.—Dosages of insecticides and gains in weight of broilers receiving insecticides in the feed for 1 week then normal feed for an additional week.

P.P.M. IN FEED	FEED/ BIRD/ DAY (G.)	TOXICANT (MG./KG./ BIRD/DAY)	WEIGHT GAIN IN LB./BIRD		
			Treated Feed	Normal Feed	Total Gain
		Bayer 22408			
0	55.0	0.0	0.08	0.19	0.27
50	47.8	3.2	.02	.17	.19
100	55.0	6.7	.19	.16	.35
200	50.7	13.8	.02	.09	.11
400	55.0	30.6	.12	.10	.22
800	43.6	42.4	−.10	.33	.23
		Bayer 29493			
0	53.6	0.0	0.18	0.27	0.45
50	46.9	4.6	.12	.21	.33
100	38.3	8.2	.09	.28	.37
200	23.0	10.4	−.10	.29	.19
400	7.4	7.4	−.12	.31	.19
800	3.0	6.3	−.18	.51	.33
		Bayer 34098			
0	54.3	0.0	0.05	0.26	0.31
50	54.3	3.1	.14	.27	.41
100	48.6	5.6	.20	.14	.34
200	54.3	13.9	.12	.20	.32
400	52.1	25.9	.15	.24	.39
800	48.2	55.7	.08	.23	.31
		Bayer 37342			
0	61.4	0.0	0.18	0.14	0.32
50	57.8	3.9	.17	.26	.43
100	54.3	6.6	.15	.31	.46
200	46.4	12.9	−.12	.37	.25
400	24.6	14.7	−.08	—	—
800	23.4	26.1	−.50	—	—
		Butonate			
0	64.9	0.0	0.23	0.22	0.45
50	60.2	6.5	.22	.24	.46
100	60.2	11.6	.22	.26	.48
200	60.6	26.3	.22	.26	.48
400	61.0	53.5	.24	.20	.44
800	60.9	98.2	.20	.22	.42
		Ronnel			
0	65.0	0.0	—	—	0.55
50	60.2	3.4	—	—	.66
100	64.3	8.0	—	—	.48
200	61.6	14.2	—	—	.42
400	64.2	30.9	—	—	.64
800	59.6	58.9	—	—	.40
		Ruelene			
0	64.9	0.0	—	—	0.48
50	58.7	3.7	—	—	.37
100	60.8	7.6	—	—	.44
200	62.6	14.7	—	—	.57
400	61.6	28.6	—	—	.55
800	61.7	58.7	—	—	.59

Table 2.—Mortality of house fly larvae placed in feces from broilers fed certain organophosphates in the diet.[a]

P.P.M. IN FEED	PER CENT MORTALITY OF 3-DAY-OLD HOUSE FLY LARVAE							
	Days Broilers on Contaminated Feed				Days After Return to Normal Feed			
	1	3	5	7	1	2	3	7
				Bayer 22408				
50	100	100	92	100	0	0	2	7
100	100	100	95	100	0	0	0	21
200	100	100	100	100	42	0	0	9
400	100	100	100	100	67	0	0	2
800	100	100	100	100	92	81	0	12
				Bayer 29493				
50	100	100	73	100	3	0	6	1
100	100	100	100	100	17	5	8	23
200	100	100	100	100	44	1	11	0
400	100	100	100	100	66	0	0	0
800	100	100	100	100	100	0	0	0
				Bayer 34098				
50	100	100	100	64	12	0	27	31
100	34	100	100	100	12	19	77	16
200	100	100	100	100	23	0	12	21
400	100	100	100	100	63	33	17	0
800	100	100	100	100	98	0	34	0
				Bayer 37342				
50	100	100	100	93	0	0	12	2
100	100	100	100	100	0	0	0	4
200	100	100	100	100	61	0	16	0
400	100	100	100	100	27	0	14	0
800	100	100	100	100	100	100	62	50
				Butonate				
50	7	10	2	0	0	0	0	0
100	7	6	0	2	0	0	0	0
200	6	3	0	8	0	0	0	0
400	10	5	0	0	0	0	0	0
800	6	8	7	6	0	0	0	0
				Ronnel				
50	65	74	14	13	0	0	0	0
100	94	100	87	44	17	20	15	0
200	100	100	100	100	15	17	18	0
400	100	100	100	100	22	26	5	0
800	100	100	100	100	100	42	46	0
				Ruelene				
50	12	26	8	0	0	0	0	0
100	11	27	2	0	0	0	0	0
200	24	3	15	5	0	0	0	0
400	7	0	2	0	0	0	0	0
800	34	18	39	38	30	29	29	19

[a] Mortality based on the number of larvae out of 50 that failed to emerge as adults.

Table 3.—Per cent mortality of house fly larvae placed in feces from broilers fed Bayer 22408 in the diet.[a]

P.P.M. IN FEED	DAYS BROILERS ON CONTAMINATED FEED				DAYS AFTER RETURN TO NORMAL FEED			
	1	3	5	7	1	2	3	7
3-Day-Old Larvae								
Bayer 22408								
10	44	32	48	42	0	0	0	0
25	90	90	94	66	2	0	0	0
50	100	100	100	72	20	29	20	18
Bayer 22408 Polymer								
10	10	12	0	0	0	0	0	0
25	96	100	77	88	0	0	15	0
50	100	100	100	100	0	0	12	0
1-Day-Old Larvae								
Bayer 22408								
10	65	77	100	92	47	54	40	0
25	100	100	100	98	46	44	40	20
50	100	100	100	98	69	66	65	32
Bayer 22408 Polymer								
10	2	60	86	97	38	20	30	20
25	100	100	100	97	34	49	64	21
50	100	100	100	100	55	49	47	23

[a] Mortality based on the number of larvae out of 50 that failed to emerge as adults.

and from previous work (Boyd & Arthur 1960, Buttram & Arthur 1961a) it was determined that a large percentage of the administered dose was eliminated intact in the feces. A comparison of the effectiveness of Bayer 22408 against 1- and 3-day-old house fly larvae at 10, 25, and 50 p.p.m. formulated as a polymer, or mixed directly with the feed, is given in table 3. The resulting data at the 50 p.p.m. level (table 3) substantiate earlier results (table 2). Polymer and nonpolymer formulations of Bayer 22408 were equally effective against house fly larvae. The 10 p.p.m. level of either formulation of Bayer 22408 in the feed was less effective than the 25 p.p.m. level against house fly larvae, but 1-day-old larvae were more susceptible to this compound than 3-day-old larvae. This ineffectiveness of the polymer formulation as compared with the nonpolymer was further evidenced by administering both formulations to broilers in capsules at 40 mg. per kg. As with the nonpolymer formulation, the polymer administered in the capsule was effective against larvae in the feces for only 2 days after cessation of the therapeutic dose.

DISCUSSION.—Feed consumption, gains in weight, residues, and selective toxicity are important factors to consider in the insecticide feed additive approach to house fly control. Feed containing Bayer 29493 at 50 to 800 p.p.m. had an obnoxious odor and the broilers offered this feed refused to eat as readily as birds offered untreated feed. The decreased feed consumption was reflected in negative gains in weight of the broilers (table 1).

There was also a decrease in feed consumption of birds receiving Bayer 37342 in the diet at 200 p.p.m. or higher. Unpalatability of the feed rather than some abnormal physiological effect of the compounds probably accounted for the negative gains in weight and mortalities. Ronnel had no adverse effect on gains in weight (table 1), but negative gains in weight have been reported for 1-week-old chicks receiving a single oral dose of ronnel at 800 mg. per kg. (Sherman & Ross 1959). However, in subsequent work by Sherman & Ross (1960), ronnel and Dow ET-15 were the only two materials of the six tested that caused no reductions in weight or decrease in egg production of hens on a 29-week dietary intake of these materials.

Ruelene (Brady & Arthur 1960) and butonate (Arthur & Casida 1959) are highly toxic to the house fly adult when applied topically, but the biological instability of the compounds very likely accounts for their ineffectiveness against house fly larvae (table 2). In detailed studies made on the stability and metabolism of radiophosphorus Reulene incorporated in the diet of laying hens (Buttram & Arthur 1961b), only trace amounts of intact Ruelene were recovered from the feces. Since Dipterex was highly effective for control of house fly larvae in poultry droppings (Sherman & Ross 1959, 1960), the possibility existed that butonate would perform likewise, but phosphonate hydrolysis probably preceded carboxylic ester hydrolysis and rendered the metabolites nontoxic.

Bayer 22408 was formulated as a polymer in an attempt to reduce intestinal absorption, to decrease the quantity of residues in internal tissues, to protect the phosphorothioate from enzymatic degradation, and to force a larger percentage of the administered insecticide to be eliminated in the feces. It was anticipated that the polymer formulation might be more effective than Bayer 22408 alone for controlling house fly larvae, but in these experiments there was no difference in the effect of the two formulations on 1- or 3-day-old larvae. The mechanical process of powdering the polymer may have decreased the protective action of the polymer constituents.

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Carbon Disulfide Effectiveness on Flour Beetles in Relation to Temperature and Exposure Time[1]

M. GOVINDAN[2] and L. K. CUTKOMP,
Department of Entomology and Economic Zoology, University of Minnesota, St. Paul

ABSTRACT

The influence of posttreatment temperatures and time of exposure have been studied using the fumigant carbon disulfide. Flour beetle adults, *Tribolium confusum* Jacquelin du Val and *T. castaneum* (Herbst) were tested. The temperature effects were also studied during fumigation and prefumigation periods, but greatest attention was given to the nonlinear responses resulting from different environmental temperatures following fumigation.

The results agree with most previous research in showing a positive linear relationship between treatment temperatures and per cent mortality. Narcosis of the insects followed the same relationship and in addition, the presence of a fumigant-tissue complex corresponded to the mortality. Posttreatment temperatures produced dissimilar results in that the greatest effects occurred at 5° and 35° C. This was evident with mortality, narcosis, and presence of the fumigant in tissues. Retention of carbon disulfide was determined by reacting diethylamine and cupric acetate and measuring colorimetrically at 430 millimicrons. The determination appeared to involve a carbon disulfide-induced material in the insect tissues.

An explanation for the nonlinear response occurring after post-fumigant temperatures undoubtedly includes both biological characteristics and physico-chemical properties of the fumigant. Evidence indicates the persistence of a fumigant-tissue complex at higher posttreatment temperatures.

The effectiveness of a volatile pesticide, particularly a true fumigant, can be greatly influenced by the temperature. The temperature effects are also related to a second factor, that of exposure time to the fumigant.

An earlier study (Pradhan & Govindan 1954) had dealt with temperatures at different periods of the toxicity test. This study, and a few others, particularly Sun (1947), had given critical attention to treatment temperatures and posttreatment temperatures which need to be evaluated separately. The interaction of duration of fumigant exposure with temperature effects were considered by Pradhan and Govindan.

The present study has gone into more detail of these factors and features of insect biology, using one fumigant, carbon disulfide, and the flour beetles, *Tribolium confusum* Jacquelin du Val and *T. castaneum* (Herbst). The differences in activity and mortality are correlated with retention of the fumigant.

MATERIALS AND METHODS.—The fumigation apparatus was first described by Pradhan & Govindan (1954). It consisted of (1) a 12-liter Florence flask in which a stock of the fumigant-air mixture was prepared; (2) 200-milliliter Florence flasks for insect fumigation; (3) gas burette; (4) vacuum pump; (5) manometer, and (6) calcium chloride bottle.

The method of fumigation was accomplished in three steps. First, preparation of the stock fumigant-air mixture in the large (12-liter) flask; second, transfer of the vaporized fumigant through the gas burette; and third, fumigation of insects in the small (200-ml.) flasks.

The stock fumigant was prepared by placing a sealed glass ampule containing a measured quantity of carbon disulfide in the large flask, along with a piece of wire gauze used as a stirrer. After a partial evacuation the glass ampule was broken by a gentle jerk of the flask. The flask was shaken vigorously, admitting small amounts of air at intervals until the pressure was equalized to that outside.

The transfer of the vaporized air-fumigant mixture was accomplished by connecting the large flask outlet to the gas burette and lowering the leveling column until the desired volume of fumigant was measured.

The insect fumigation flask was prepared with perforated gelatin capsules containing insects. It was then connected to the vacuum pump for partial evacuation and connected to the gas burette where a known volume of fumigant approximately equal to the concentration in question was introduced by manipulating the leveling column of the gas burette. The stopcock was turned and the fumigating flask disconnected and stoppered with a rubber cork.

The fumigant concentration was calculated as follows:

$$C = \frac{M \times V \times 1000}{V_s \times V_f}$$

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[2] Daiva sigamany Mudaliar Road, Lakshmipuram, Royapettah, Madras, India.

where,

C = concentration in milligrams of fumigant per liter in the insect fumigation flask
M = weight (milligrams) of fumigant placed in and vaporized in stock flask
V = volume (milliliters) of fumigant-air mixture introduced in the fumigation flask
V_s = volume (milliliters) of the stock flask
V_f = volume (milliliters) of the fumigation flask

The gas burette had sufficient volume for three fumigation flasks (65 milliliters). Following each buretting the pressure in the stock flask was equalized with the atmosphere. Thus, the value M in the formula was changed with each buretting and the value of V had to be recalculated.

Temperatures were controlled during fumigation treatment and following treatment (posttreatment). The fumigation flasks and recovery vessels were placed in thermostatically controlled cabinets. Temperatures used were 5°, 10°, 15°, 20°, 25°, 30°, and 35° C. A constant relative humidity of 45% to 50% was maintained using desiccators charged with proper concentrations of potassium hydroxide or potassium nitrate.

The fumigant used throughout was carbon disulfide, analytical reagent.

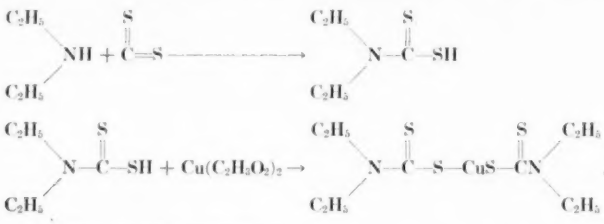
The primary test insect was the confused flour beetle, *Tribolium confusum*. Stock cultures of known age were reared in a mixture of branless wheat flour supplemented with 5% brewer's yeast (Shepard 1943). The adults were of mixed sexes 4 weeks old at testing time. Fewer tests were conducted with the red flour beetle, *Tribolium castaneum*, merely to determine whether a similar trend in results occurred. During fumigation 30 insects per replicate were kept inside a gelatin capsule size #0. The capsules had been uniformly perforated with holes.

Following fumigation the insects were released in tin boxes or open vials and kept under postfumigation conditions until the mortality determination, usually 7 days. Food was given in most experiments and mortality counts made daily. One comparative test was made in the absence of food.

Statistical treatment of mortality data selected as most appropriate was a method to determine day-to-day results (and trends) for each of the three replicates from each of the seven postfumigation treatments. Orthogonal polynomials were used to determine whether linear, quadratic, or cubic trends occurred. The analysis of variance was thus used to determine which trend was most important. A less informative type of analysis would have been one based upon cumulative totals on a given day. This seemed undesirable because the day-to-day trend would be ignored. Furthermore, one would have seven analyses for each comparison and each analysis would contain some of the same information as the prior analysis.

Chemical analyses of carbon disulfide were made to relate to insect mortality and narcotic effects.

The determination of CS_2 in biological tissues had been made by McKee (1941) and Strittmatter et al. (1950) using vertebrates. Their methods were adapted for use on a microanalytical basis. The reagents, 0.5% diethylamine and 0.001% cupric acetate, dissolved in 95% ethanol, react with CS_2 to form cupric diethyl thiocarbamate, a yellow compound with maximum absorption reading at 430 millimicrons. The reaction is as follows:



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Insects were macerated to yield an extract for chemical analysis. Usually 10 milligrams were extracted with 0.5 ml. ethanol. Analysis was carried out by adding 2 ml. of insect extracts and CS_2 to the sample and adding 4 ml. of 95% ethanol.

Fortification of fumigated insects with known quantities of carbon disulfide was necessary. The results obtained from this procedure are illustrated in figures 1 and 2. The absorption curve for carbon disulfide alone is given in figure 1. Additional curves illustrate the increased optical density when fumigated insect extracts were used. Figure 2 illustrates the increased optical density caused by the addition of increments of carbon disulfide. Further experiments showed that heat-killed insects did not increase optical density. Wheat fumigated with carbon disulfide also had no detectable effect on the colorimetric test.

The analytical procedure was checked further by tests to show that the alkaline condition produced by the reaction with diethylamine was not responsible for increased optical density. This was done by substituting sodium hydroxide to produce an equivalent hydrogen ion concentration. Optical density was also unaffected by the addition of cupric acetate alone.

RESULTS.—The results are presented in three parts, (a) mortality as related to temperature and time of exposure, (b) insect movements as related to temperatures, and (c) insect retention of carbon disulfide as related to temperatures.

(a) *Mortality as Related to Temperature and Time of Exposure.*—Preliminary experiments were made to determine the LD_{50} of carbon disulfide to *Tribolium confusum* for a 24-hour exposure at 25° C. The 5-day mortality was determined using 90 insects for each of six doses. The LD_{50} was determined to be 32 mg. per liter (31.4 to 32.4 mg./l at 95% confidence limits).

The LD_{50} value served as a basis for future experiments. Thus, using 32 mg./l and a 24-hour exposure, tests were conducted as given in the diagram:

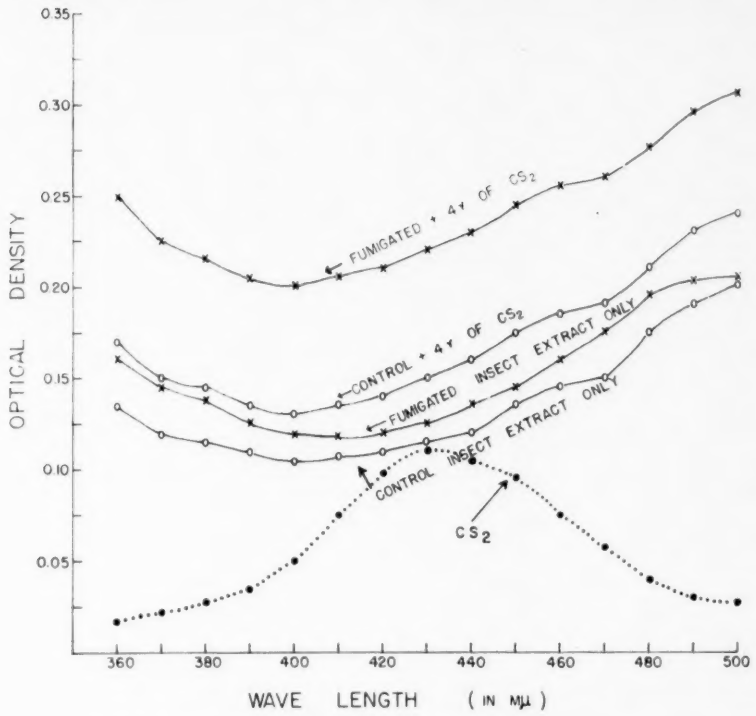

FIG. 1.—Absorption spectra of ethanol extracts of control insects and fumigated insects with and without fortification by CS_2 to extracts. Color test with diethylamine and cupric acetate. The absorption curve is shown for CS_2 alone without extracted material.

Prefumigation temperature in ° C.	*Fumigation Temperature in ° C.*	*Postfumigation Temperature in ° C.*
	10	10
		25
		32
25	25	10
		25
		32
	32	10
		25
		32

The results from the above tests showed a positive temperature effect for mortality during fumigation at all three posttreatment temperatures of 10°, 25°, and 32° C. The posttreatment (fumigation) temperature also indicated a positive effect at treatment temperature of 25° C., but the posttreatment temperature did not show any clear-cut trends at 10° or 32° C. This was true because of low per cent mortality at 10° C. and high per cent mortality at 32° C.

Thus, a valid comparative effect of posttreatment temperatures was made by reducing the time of exposure at the high treatment temperature of 32° C. A 12-hour exposure was adequate. The linear positive temperature effect, quite pronounced during treatment, was not in evidence in the posttreatment period (fig. 3). Instead, the greatest toxic effect occurred at 10° and 32° C. with the 25° posttreatment effect being least.

The influence of posttreatment temperatures was studied in considerably more detail, using seven posttreatment temperatures, 5°, 10°, 15°, 20°, 25°, 30° and 35° C. Exposure times of 6, 8, 10, and 12 hours were made. The results are shown in figure 4, illustrating only two of the selected treatment times. The results from all exposure times were in agreement in that the extreme posttreatment temperatures (5°, 30° and 35° C.) produced a much higher mortality than intermediate temperatures. Statistical analyses revealed a quadratic relationship over the temperature range instead of a linear or cubic relationship.

The posttreatment time at the chosen temperatures was then reduced from 7 days to 24 hours, with all test insects being returned to 25° C. This did not alter the trend of higher mortality at the extreme temperature, although the overall intensity of effect was lessened (figure 4). *Tribolium castaneum*, although more sensitive to the fumigant, was affected by posttreatment temperatures similarly (figure 5). Perhaps the best graphic comparison (using *T. confusum*) of three posttreatment temperatures is given in figure 6 where the duration of posttreatment temperatures was 1 day, 2 days, and 3 days (fig. 6).

The rather unusual mortality trend (increased by temperature extremes) attributed to posttreatment temperature effect could conceivably be influenced by the

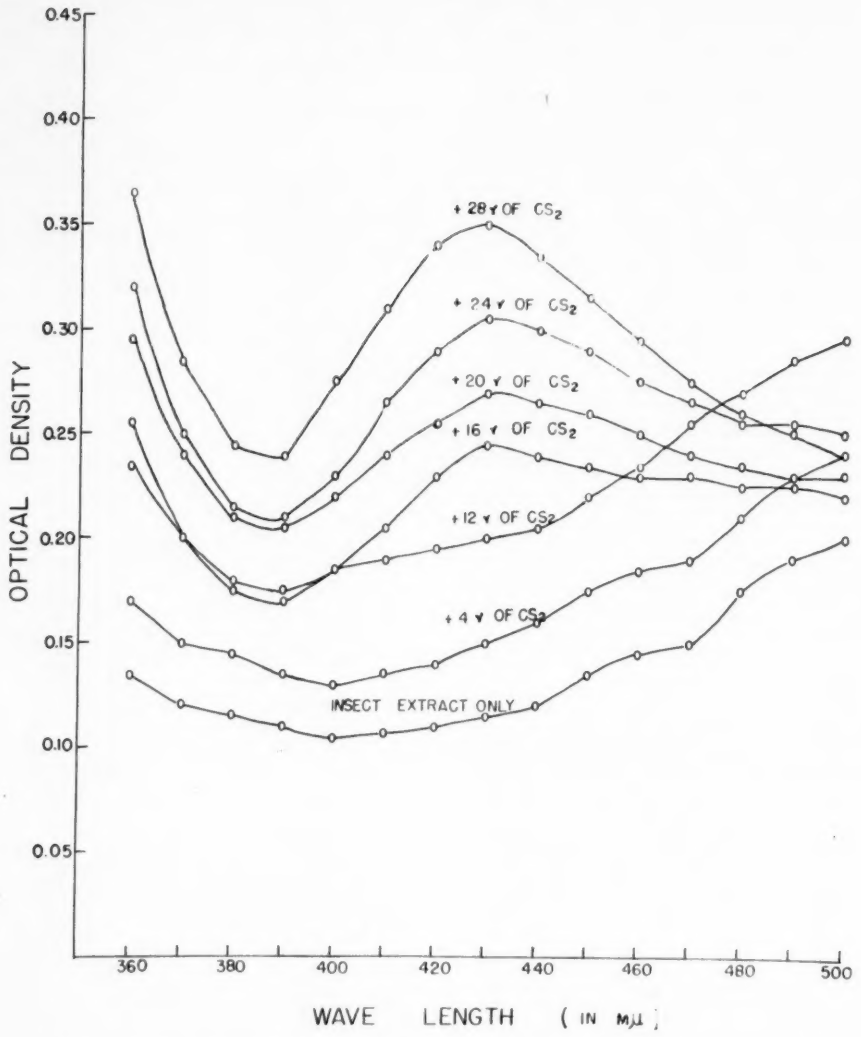

FIG. 2.—Absorption spectra of ethanol extracts of control insects and extracts with additions of increasing concentrations of CS_2. Absorption maxima is 430 mμ.

rearing temperature for the insects. This was tested by rearing populations at 35° C. instead of 25° C. and tests of this population produced a very similar trend; the chief difference was evidence of a little greater susceptibility by the population reared at 35° C. (fig. 7).

(b) *Insect Movements as Related to Temperature.*—The mortality results expressed at the different posttreatment temperatures were in agreement with Sun (1947) and Pradhan & Govindan (1954). They were not in accord with all previous studies, however, and it seemed desirable to use a sensitive biological response to add to the information.

A method for determining narcosis was adopted even though the precise action of carbon disulfide is unknown. The method used by Bradbury & Armstrong (1954) on granary weevils was adopted with minor modifications. The insects were stimulated to move using light as a negative stimulant. Lack of movement was used as an index of the degree of narcosis. Following treatment at selected temperatures each group of insects was released inside a 1″-diameter circle marked on troughs of filter paper. The troughs were placed on a glass plate and exposed to light from beneath. The number of insects that failed to move beyond an outer circle of 2″ diameter within 2 hours were recorded as affected.

The first test involved treatment temperatures of 5°, 20°, and 35° C. and illustrated a positive temperature effect, as had been the case with mortality. The time of exposure to the fumigant was reduced to 2 hours for best comparisons (table 1).

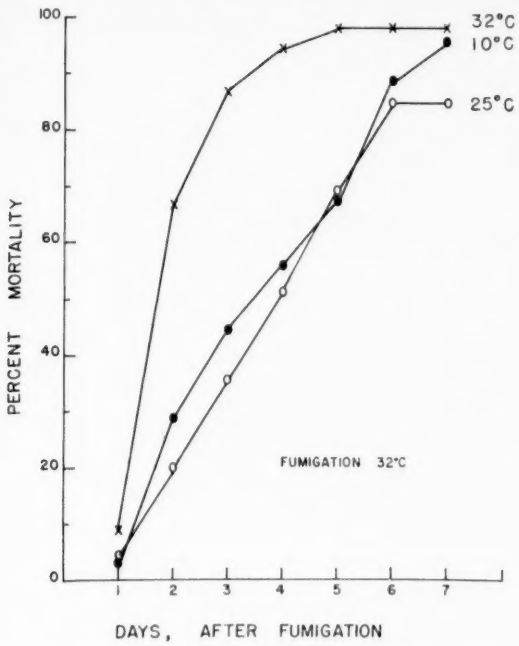

FIG. 3.—The effect of posttreatment temperatures on 7-day mortality of *T. confusum* adults fumigated with 32 mg. CS_2 per liter for 12 hours. Thirty insects were used for each of three replicates.

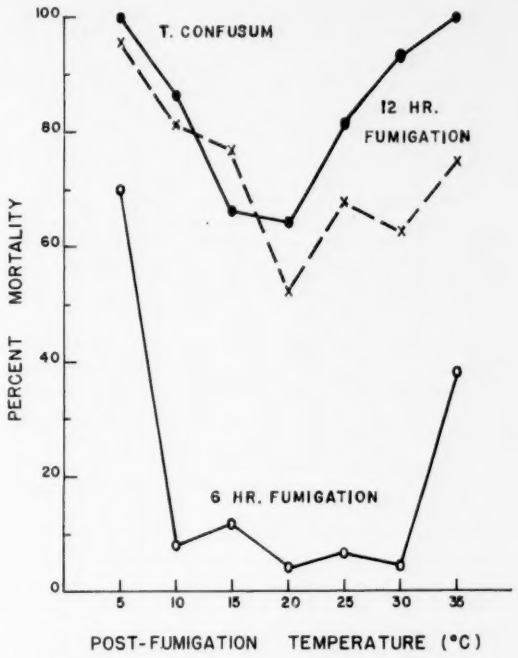

FIG. 4.—The effect of 7 posttreatment temperatures on 7-day mortality of *T. confusum* adults fumigated with 32 mg. CS_2 per liter for 6 or 12 hours. The dotted line represents data from insects having a short exposure of 24 hours to the posttreatment temperatures. The beetles were then transferred to 25° C.

The posttreatment temperature effect was of considerable interest to determine if the trend was similar to that obtained in the mortality studies. Results are presented in table 2. Thus, the trend of a higher per cent showing narcosis at 5° and 35° than at 20° C. is similar to mortality studies. Furthermore, as with mortality, the effect at 5° C. appeared somewhat greater than at 35° C.

(c) *Insect Retention of Carbon Disulfide as Related to Temperature.*—Chemical determinations of carbon disulfide as related to mortality and narcotic effects are given. These determinations, based upon the colorimetric test of McKee (1941) and Strittmatter et al. (1950) provided quantitative comparisons. Since the values were elevated, however, when fumigated insect extracts were used (see figs. 1 and 2), the material being determined is called a carbon disulfide-induced material. Determinations are of necessity based upon a standard curve using known amounts of carbon disulfide incorporated in insect extracts.

Table 1.—Per cent of *Tribolium confusum* adults which failed to move when exposed to light, after fumigation at 5°, 20° and 35° C. with 32 mg./l. of CS_2 for 2 hours.[a]

MINUTES AFTER FUMIGATION TEMPERATURE TREATMENT[b]	TREATMENT TEMPERATURE (° C.)		
	5	20	35
30	88.9	91.1	100.0
60	68.9	85.6	100.0
120	45.6	66.7	88.9

[a] Each treatment involved three replications using 30 insects each.
[b] All control insects moved in less than 30 minutes.

The results for the three treatment temperatures, 5°, 20°, and 35° C., indicated progressively greater amounts of CS_2 present in fumigated insects from low temperatures to high (see table 3). Using a treatment temperature of 35° C., the order of CS_2 retention was greatest at 5° C., intermediate retention at 35° C. and least at 20° C. These results, shown in table 3, give relative magnitudes for comparison. The results indicate that the carbon disulfide-induced material was present in the insects in the same order of relationship as mortality values, both with respect to fumigation or postfumigation temperatures. Narcotic effects followed a similar relationship. This effect is shown for both species. Greater retention is shown for *T. castaneum*. This is also related to greater susceptibility of this species.

DISCUSSION.—Studies on the effect of temperature on fumigant action have often yielded conflicting results. Much of the difficulty can be attributed to failure to clarify effects during the three periods, pretreatment, treatment, and posttreatment. The present study has shown a positive relationship with temperature during treatment (fumigation). Greater mortality at higher temperatures during treatment has been most commonly reported (Cotton 1932, Moore 1936, Pradhan & Govindan 1954, Sun 1947); some exceptions have occurred, with the negative trend usually explained on a biological basis (Sun 1947). Certainly a fumigant increases in volatility at

Table 2.—Per cent of *Tribolium confusum* adults which failed to move when exposed to light, after posttreatment for 2 days at 5°, 20° and 35° C.[a]

Minutes After Postfumigation Temperature Treatment[b]	Postfumigation Temperature (° C.)		
	5	20	35
30	100.0	85.6	96.7
60	100.0	75.6	91.1
120	100.0	61.1	84.4

[a] The insects were fumigated at 35°C. For 10 hours with 32 mg./l of CS_2. Each treatment involved 3 replications using 30 insects each.
[b] All control insects moved in less than 30 minutes.

higher temperatures and in the gaseous phase chemical reactivity is considered to be greatest. Insect respiration increases over the temperature range studied, 5° through 35° C. Thus, the expected uptake of the fumigant would be increased over the range studied. The present study has advanced evidence of this, using a colorimetric determination, and a narcotic effect, both following a similar trend.

Posttreatment effects, instead of producing a positive temperature relationship, resulted in the greatest mortality, narcotic effect, and carbon disulfide (or fumigant-complex) retention at temperature extremes. All three factors were somewhat greater at 5° C. than at 35° C., and the effects were similar for two related insect species. A similar trend as measured by mortality has been reported

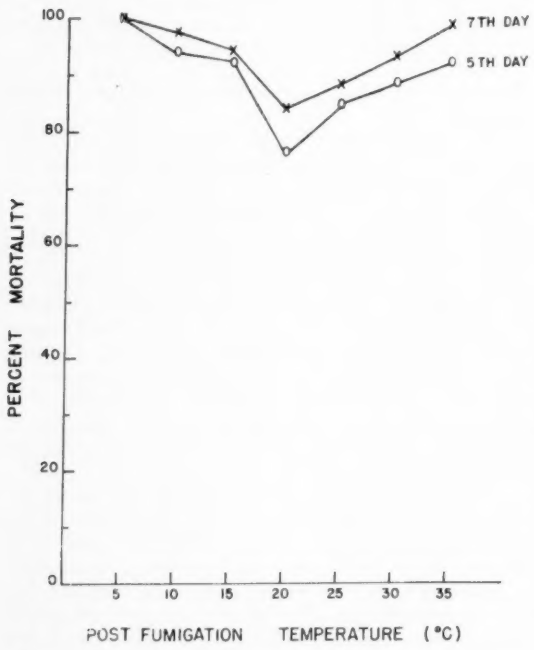

FIG. 5.—The effect of 7 posttreatment temperatures on 7-day mortality of *T. castaneum* adults fumigated with 32 mg. CS_2 per liter for 10 hours. As described for figure 4, the beetles had a short exposure of 24 hours to the posttreatment temperatures, then were transferred to 25° C.

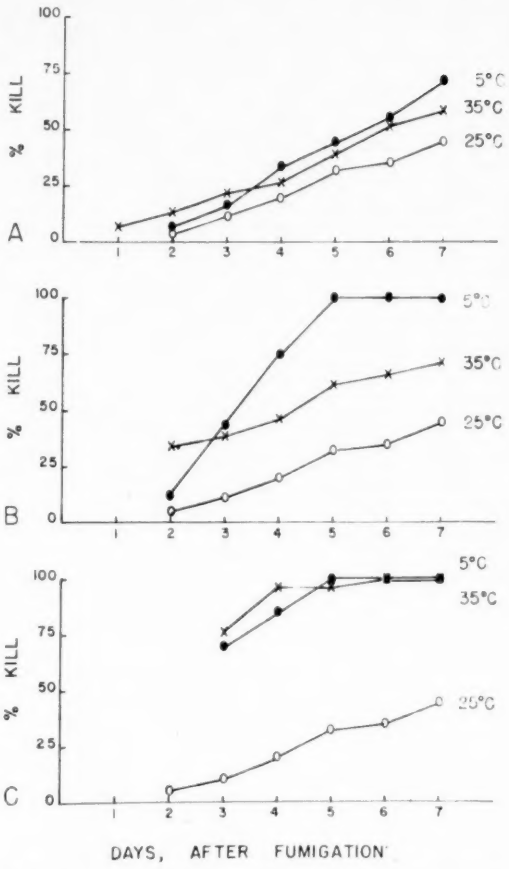

FIG. 6.—A comparison of exposure time to three posttreatment temperatures. A=24-hour exposure; B=48-hour exposure; and C=72-hour exposure. Beetles were transferred to 25° C. afterwards. Results were based on 7-day mortality figures for *T. confusum* fumigated with 32 mg. CS_2 per liter for 10 hours at 35° C.

by Sun (1947) using *T. confusum* and *Sitophilus granarius* (L.). His explanation for the greater effect at lower temperatures follows: "the chemical reaction of fumigant upon an insect is decreased as the temperature decreases; however, a low rate of desorption and prolonged action of the fumigant in the body must be considered." This

Table 3.—Amount of carbon disulfide-induced material determined from *Tribolium confusum* and *T. castaneum* for 24 hours using a concentration of 32 mg. CS_2 per liter.

Temperatures	μg. CS_2-Induced Test per Mg. Insect Extract		
	5° C.	20° C.	35° C.
T. confusum			
Fumigation temperature[a]	0.21±0.01	0.35±0.01	0.41±0.01
Postfumigation temperature[b]	0.18±0.01	0.08±0.02	0.12±0.02
T. castaneum			
Postfumigation temperature[b]	0.37±0.01	0.17±0.01	0.28±0.01

[a] Following fumigation exposure all insects returned to 25° C.
[b] All fumigated at 35° C. for 12 hours, then kept at respective postfumigation temperatures for 24 hours.

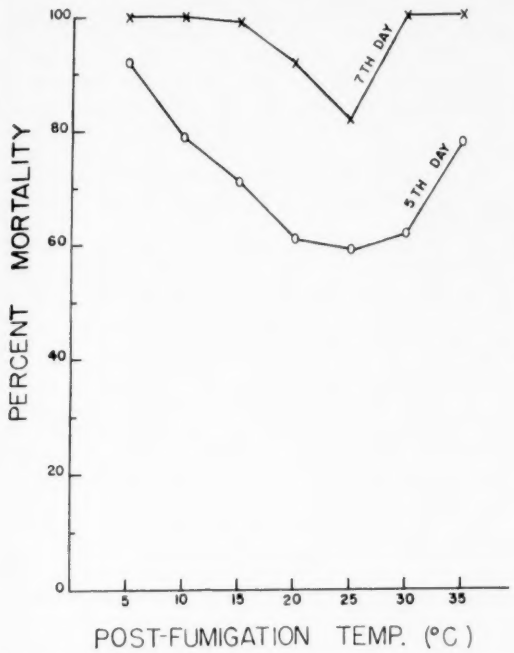

FIG. 7.—The effect of higher rearing temperature, 35° C. comparing 7 posttreatment temperatures on 7-day mortality of *T. confusum* adults. Beetles were fumigated with 32 mg. CS_2 per liter for 10 hours at 35° C. They were then subjected to the respective posttreatment temperatures for 24 hours each.

would agree with the present results which include narcosis and chemical retention. The explanation for the greater effect at the higher temperature may be more debatable. Sun has indicated that "at higher temperatures from 30° to 35° C. the temperature is so high that the chemical action of carbon disulfide upon insects may kill them in a relatively shorter time, before any sufficient amount can be eliminated through desorption, diffusion and respiration." Our evidence would not seem to support this explanation, particularly with respect to the rapid kill.

In the present study a uniform initial pick up (or uptake) of CS_2 would be expected because treatment was made at a uniform temperature, 32° C. in most comparisons. However, if a quick kill is the explanation for greater mortality at higher temperatures the study on narcosis (as measured by lack of movement) should show this effect. It did not. Also, shortening the posttreatment temperature period caused no deviation in the trend. Furthermore, higher temperatures increase CS_2 volatility and presumably desorption rates. But the colorimetric test revealed greater retention than in the moderate temperature range of 15° to 25° C. Perhaps the explanation is that more carbon disulfide was absorbed in the initial posttreatment period and CS_2 induced products formed which were retained in the tissues in a nonvolatile but biologically active state. The formation of a complex with tissues or some biological system would be expected to reduce desorption rates.

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Evaluation of New Materials for Codling Moth Control in Washington[1]

STANLEY C. HOYT[2]

ABSTRACT

Field studies were conducted during 1958, 1959 and 1960 to determine the effectiveness of several insecticides for the control of codling moth (*Carpocapsa pomonella* (L.)) in apple orchards which had had a high percentage of wormy fruit during the previous year.

Sevin® (1-naphthyl *N*-methylcarbamate) at 1 pound of 50% wettable powder per 100 gallons of water or Guthion® (*O,O*-dimethyl *S*-4-oxo-1,2,3-benzotriazin-3-(4H)-ylmethyl phosphorodithioate) at 0.75 pound gave excellent codling moth control. At a dosage of 1.5 pounds per 100 gallons of water both of these materials provided good control when the interval between sprays was extended from 21 to 28 days. Ethyl Guthion® (*O,O*-diethyl *S*-4-oxo-1,2,3-benzotriazin-3(4H)-ylmethyl phosphorodithioate) and Diazinon® (*O,O*-diethyl *O*-(2 isopropyl-6-methyl-4-pyrimidyl) thiophosphate) were not consistently as effective as

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[2] Assistant Entomologist, Tree Fruit Experiment Station, Wenatchee, Washington. Grateful acknowledgment is made to Jerrold R. Kinney for assistance in carrying out these studies.

Sevin or Guthion. Control with ethion was good in 1958 and 1959 but was poor during 1960. Trithion® (*S*-(*p*-chlorophenylthio methyl)*O,O*-diethyl phosphorodithioate) gave good control but was phytotoxic to apples. Other materials which showed considerable promise for codling moth control were dimethoate, phosphamidon, Bayer 29493 (*O,O*-dimethyl *O*-[4-(methylthio)-*m*-tolyl] phosphorothioate), and Bayer 22408 (Naphthaloximido-*O,O*-diethyl phosphorothioate).

The materials which did not provide adequate control with Ryania (ground stems of *Ryania speciosa*), were: Dilan (2-nitro 1,1-bis(*p*-chlorophenyl)propane and butane mixture (1:2 ratio)), Kepone® (decachlorooctahydro-1,3,4-metheno-2H-cyclobuta [cd] pentalen-2-one), and DDT. The addition of either parathion or malathion to DDT resulted in good control.

In the State of Washington the codling moth (*Carpocapsa pomonella* (L.)) normally has two generations a year, but in the warmer years a partial third generation may develop. The first moths of the overwintering generation usually appear about May 10. Flights continue from that time until the end of June or early July. The first generation moths appear from the latter half of July until early September. Larvae are present in the fruit from about May 20 until early October. Control can generally be accomplished by the application of two or three cover sprays against the first brood worms. Synthetic organic insecticides have made possible this simple and highly effective program of control. Reports from apple-packing organizations show that fruit from most orchards contain less than 0.5% wormy apples.

Despite these low populations the codling moth remains an important pest. Its potential destructive ability necessitates the use of expensive preventive programs which induce other orchard problems by the destruction of parasites and predators.

Mites are currently the major problem in Washington orchards. As a consequence, much of the emphasis on experimental work has been shifted to the control of mites. One aid to mite control could come from the use of materials for codling moth control which would not destroy mite predators or result in a mite buildup. Ryania (ground stems of *Ryania speciosa*) has been used by Clancy & McAlister (1956) and Pickett (1959) as a selective insecticide for codling moth control. These authors report good codling moth control and little or no toxicity to predaceous mites with this material.

Because of the development of codling moth resistance to DDT in Western States as reported by Hamilton (1956), Madsen & Hoyt (1958), and Barnes (1958), it seems probable that this species eventually will develop a resistance to other organic insecticides. Information on alternate materials will be needed if the codling moth develops resistance to materials now giving control.

MATERIALS AND METHODS.—Tests were conducted during 1958, 1959, and 1960 in orchards where a high percentage of wormy fruit had occurred the previous year. In the 1958 and 1959 tests, three cover sprays were applied against the first-brood larvae and one cover spray against the second-brood larvae. The number of sprays used in the 1960 tests is described below.

In all cases the first cover spray and cover sprays against the second-generation worms were timed by the use of bait pans which indicated the presence of adult moths. The second cover spray was timed 21 days after the first, and the third cover spray 21 days after the second in all tests except 1960-3. Minor variations in the 21-day interval occurred in some cases owing to weather unfavorable for spraying on the designated day. High pressure equipment and orchard guns operating at 600 p.s.i. were used to spray all plots except test 1958-1.

Untreated check trees were not used in the 1958 and 1960 tests since the lack of a treatment leads to excessive fruit loss.

Test 1958-1.—An orchard of Yellow Newton apples at White Salmon on the lower Columbia River was selected for this test. Over 33% of the fruit from this orchard was wormy in 1957 when a DDT program was used. One-acre plots replicated twice were established. To prevent extensive fruit loss in the event that the codling moth was resistant to DDT, DDT plots were reduced in size to 8-tree replications. All sprays were applied with air carrier equipment using approximately 750 gallons of spray per acre. The sampling was done by examining the dropped fruit from under 25% of the trees just prior to harvest, and 25 boxes of picked fruit per replication at harvest.

Test 1958-2.—A second series of plots was established near Omak, Washington, in an orchard of large Winesap apple trees with a few Red Delicious and Jonathan trees. This orchard was adjacent to an abandoned orchard and as a result of faulty spray practices the grower experienced difficulty in controlling codling moth during 1957. Four-tree plots replicated three times in a randomized block design were established, and approximately 1,400 gallons of spray per acre were applied. The treatments were evaluated by examining the dropped fruit from 25% of the area under each tree plus 600 apples per replicate at harvest for worms or stings.

Tests 1959-1 and 2.—Two orchards with heavy infestations of the codling moth in the previous year were used for the 1959 tests. Both orchards were composed primarily of Winesap trees with a few Delicious and Jonathan trees. In the Malaga orchard (1959-1) single-tree plots replicated five times were established, but one entire replication was removed from the test owing to a lack of infestation. In the Wenatchee orchard (1959-2) single-tree plots replicated four times in a randomized block design were established. The spray applied in each test averaged 800 gallons per acre. Samples of 150 apples per tree were examined for larval entries or stings. The dropped fruits from under each tree were collected just prior to harvest, but no more than 50 per tree were examined. The remainder of the sample was examined at harvest.

Test 1960-1.—An orchard of large Delicious apple trees was used for the first of the 1960 tests. Single-tree plots replicated four times were established in a randomized block design. Approximately 1,200 gallons of spray per acre were used on these large trees.

Three cover sprays were applied against the first brood worms. Since further moth flights were not observed in this orchard, no second brood sprays were applied. Samples of 200 apples per tree were examined for worms or stings. Since very few fruit had dropped, all drops were examined the day before harvest; the remainder of the sample was examined at harvest.

Tests 1960-2 and 3.—The final series of plots was established at Wenatchee in a Delicious apple orchard

Table 1.—Results of field tests for the control of codling moth on apples in Washington. 1958–60.

Material and Per Cent Active Ingredient	Rate per 100 Gal.[a]	Per Cent Worms and Stings (in parentheses) for Each Test During 1958–60						
		1958		1959		1960		
		1	2	1	2	1	2	3
Bayer 22408, 50	1.5					2.1 (.4)		
Bayer 29493, 25	2						0.9 (2.9)	
DDT, 50	2	4 (7.4)	8.4 (3.0)		8.8 (2.0)	9.1 (.6)		
DDT, 50+parathion, 25	2		2.5 (1.8)	2.1 (3.4)	2.5 (.7)			
	1							
DDT, 50+malathion, 25	2				1.0 (0)			
	2							
DDT, 50+toxaphene, 40	2							
	2					7.4 (.4)		
Diazinon, 25	2	2.4 (8.9)	0.5 (1.5)	1.0 (6.4)	0.5 (1.7)		4.2 (9.8)	4.1 (9.8)
Dilan, 25	2					5.7 (.5)		
Dimethoate, 50	2				0.2 (.8)			
	1				0.8 (1.7)			
Dimethoate, 25	1.5					2.2 (1.0)	7.1 (8.7)	10.1 (9.5)
Ethion, 25	2	0.05 (4.4)	1.1 (2.2)	1.8 (3.9)	0.3 (1.7)		19.9 (4.4)	
Ethion, 25+oil, 98, emulsifiable	2				0.8 (.7)			
	2 qt.							
Ethyl Guthion, 25	1.5				0 (.5)			
	1			7.5 (4.0)	0.3 (.2)			
Guthion, 25	1.5	0.05 (.6)	1.1 (.7)					0.3 (1.6)
	1.25					0.3 (0)	0.8 (.8)	
	1			3.3 (3.5)				
	0.75					0.5 (0)		
Guthion, 25+ Ryania, 50	0.75					0.8 (.4)		
	3							
Kepone, 50	2		5.4 (2.4)					
Kepone, 25	2			8.2 (5.4)				
Phosphamidon, 40	0.75					1.5 (.3)		
Ryania, 50	6					6.5 (0)		
Sevin, 50	1.5	0.03 (0.6)	0.2 (.6)	1.4 (2.8)				2 (1.2)
	1			1.6 (3.4)	0 (.2)	1.0 (.5)		
Sevin, 25–ethion, 17	2					.8 (.1)		
Trithion,[b] 25	2		0.9 (0.9)					
Untreated	—			17.4 (6.9)	31.7 (3.7)			

[a] All rates are in pounds of wettable powder unless otherwise noted.
[b] Trithion in the first two cover sprays only. Two pounds of DDT plus 1 pound of parathion per 100 gallons of water were substituted for Trithion in the third and fourth cover sprays.

which had an extremely high percentage of wormy apples during 1959. The plot design was the same as in the previous test, and the amount of spray used per acre averaged 1,000 gallons. Six of the materials were applied at 21-day intervals (1960-2) using three applications against the first generation larvae and two against the second generation. The remaining four materials (1960-3) were applied twice against the first generation larvae with an interval of 28 days between sprays. Two sprays were also applied to control the second-generation larvae, and both of these sprays were timed by the appearance of moths in bait pans. Two-hundred and fifty apples per tree were examined for worms or stings, including the dropped fruit from 25% of the area under each tree.

The following list contains the chemical definitions of the materials tested which do not appear in the list of common names of insecticides (Smith 1959).

Sevin®—1-naphthyl *N*-methylcarbamate
Guthion®—*O,O*-dimethyl *S*-4-oxo-1,2,3-benzotriazin-3-(4H)-ylmethyl phosphorodithioate
Ethyl Guthion®—*O,O*-diethyl *S*-4-oxo-1,2,3-benzotriazin-3(4H)-ylmethyl phosphorodithioate
Ryania—ground stems of *Ryania speciosa*
Diazinon®—*O,O*-diethyl *O*-(2 isopropyl-6-methyl-4-pyrimidyl)-thiophosphate
Oil, Volck Supreme—98%, highly paraffinic oil. UR 93 Viscosity 100° F., SSU-142
Bayer 29493—*O,O*-dimethyl *O*-[4-(methylthio)-*m*-tolyl] phosphorothioate
Bayer 22408—Naphthaloximido-*O,O*-diethyl phosphorothioate
Dilan—2-nitro 1,1-bis (*p*-chlorophenyl)propane and butane mixture (1:2 ratio)
Kepone®—decachlorooctahydro-1,3,4-metheno-2H-cyclobuta [c d] pentalen-2-one
Trithion—*S*-(*p*-chlorophenylthio methyl) *O,O*-diethyl phosphorodithioate

RESULTS AND DISCUSSION.—The results of all the tests are summarized in table 1. Sevin provided excellent control of the codling moth at dosages of 1 to 1.5 pounds of 50% wettable powder per 100 gallons of water. When the interval between applications of Sevin was extended to 28 days (table 1, 1960-3) good control was still provided. Multiple applications of Sevin brought about extreme increases in mite populations. Previous experience has shown that this problem can be overcome by adding a suitable acaricide to one of the early cover sprays. The Sevin-ethion combination controlled the codling moth, but insufficient data are available to determine whether this combination overcame mite increases which occurred following the use of Sevin alone.

The codling moth control provided by Guthion was excellent at dosages as low as 0.75 pound of 25% wettable powder per 100 gallons of water or intervals as long as 28 days between applications. Although this material did not control the McDaniel spider mite (*Tetranychus mcdanieli* McGregor) at rates of 1.5 pounds per 100 gallons of water, its use did not result in extreme increases in population. Where Ryania was added to Guthion the dosage of Guthion alone was sufficient to provide excellent control of the codling moth. It was not possible to determine if Ryania improved the control. The ethyl analog of Guthion gave more variable control than Guthion. In one orchard (test 1959-1) poor control resulted from applications of the ethyl analog.

Although Ryania was shown to be effective for codling moth in New York and Indiana (Hamilton & Cleveland 1957), West Virginia (Clancy & McAlister 1956), and Nova Scotia (Pickett 1959), satisfactory control was not obtained with this material in Washington, probably due to the longer intervals between sprays.

Diazinon has been tested for several years for codling moth control. It remains of considerable interest because of the excellent control of aphids provided when using it in a codling moth program. The results of tests with Diazinon listed in table 1 indicate more variable control than that provided by some materials. In several of the tests the high percentage of stings found in fruit sprayed with this material indicated a short residual action. Madsen & Falcon (1960) also report short residual effectiveness for Diazinon.

The control provided by ethion during 1958 and 1959 was excellent and indicated that this was a very promising material. However, during the 1960 trials ethion gave very poor control despite five applications. The material used in the 1960 test contained slightly less than 25% active ingredient, but it is doubtful if this lower percentage of the active ingredient can be wholly responsible for the poor control obtained. The addition of oil to ethion did not appear to enhance codling moth control, but did result in some mite suppression. This combination would be of questionable value owing to the incompatibility of oil with late-season fungicides for apple scab or bull's eye rot control.

Fifteen years ago, O'Neill & Lipovsky (1946) reported good control of the codling moth in north central Washington with DDT at rates of 1 pound or less of 50% wettable powder per 100 gallons of water. In the current study in the same area DDT did not provide adequate codling moth control at a 2-pound dosage. This suggests that the codling moth has developed resistance to DDT, although not so extreme as that reported by Madsen & Hoyt (1958) or Madsen & Falcon (1960). In those orchards where the previous years' results implied severe DDT resistance, a well-timed program with thorough application resulted in less than 10% wormy fruit. The high populations the previous year were probably due to poor application of sprays or improper timing.

The addition of either parathion or malathion to DDT resulted in good control. The addition of toxaphene did not improve the control appreciably over that obtained with DDT alone. Applications of Dilan or Kepone resulted in poor control comparable with that obtained with DDT.

Dimethoate gave excellent worm control during 1959, but the 2-pound rate caused a russeting of 1.3% of the apples. The dosage was decreased during the 1960 trials, and the control was inconsistent. This compound is of particular interest because it is not only promising for codling moth control, but provides excellent control of the apple aphid (*Aphis pomi* De Geer) and the woolly apple aphid (*Eriosoma lanigerum* Hausmann).

Of the remaining materials tested, phosphamidon, Bayer 29493 and Bayer 22408 showed considerable promise. Trithion also showed promise for control of the codling moth, but was dropped from the testing program because it caused fruit and foliage injury.

Studies on the effects of most of these compounds on mite populations or on predators and parasites have not progressed far enough to determine whether they will be useful as selective insecticides in a program of integrated control. It is suspected, however, that the broad spectrum of insecticidal activity of most of these materials will not permit their use in this type of program.

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Cotton Fiber Quality as Related to Some Cotton Insect Control Programs and Time of Fruit Setting[1]

LORRAINE PHILLIPS,[2] A. D. OLIVER[3] and LUCILLE FINLEY[4,5]

ABSTRACT

Removal of fruit from the cotton plant for 0, 3, and 7 weeks after fruiting began caused differences in certain fiber properties for the 7-weeks' removal only. Fiber quality as influenced by fruit removal was apparently associated with the variety.

Fiber quality, other than fineness, of cotton produced by plants which received insecticide applications for protection from cotton insects beginning at plant emergence and continued to maturity did not differ from that produced by plants which received insecticides applications for control of cotton insects only after the boll weevil infestation reached 25% or where no insecticides were used. All treatments receiving insecticide applications yielded finer fiber regardless of application schedule.

The effect of insect infestations on cotton yields is well known. Information relative to the effect of insect infestations or use of insecticides on the quality of cotton fiber is limited. During the last decade an insect control program for cotton referred to as "early season" control has been increasingly recommended in some areas. Proponents of early season cotton insect control program claim that cotton fiber of a higher quality results.

Increased awareness of the demand for fiber specifications by manufacturers reflects the importance of any practice that may influence fiber quality. Fiber length, strength and fineness are physical properties in current use to determine quality of raw cotton fiber. This study was made to determine if various insect control practices and time of fruit setting, as determined by defloration, affect fiber quality.

PROCEDURE.—Cotton fiber used was obtained from defloration experiments conducted at St. Joseph and Winnsboro, Louisiana in 1956, 1958, and 1959, and from an insect control experiment, using insecticides, conducted in 1959 at St. Joseph.

Defloration Experiment.—Fiber obtained from treatments in which the fruit was removed for 0, 3 and 7 weeks, respectively, after the cotton began to fruit was used as representative of fiber from early, mid-, and late-season fruiting. The time of fruit set was controlled by removing by hand all squares match-head size and larger at weekly intervals. Insects in all plots were effectively controlled by applications of toxaphene, DDT, methyl parathion, and Guthion®(*O,O*-dimethyl *S*-4-oxo-1,2,3-benzotriazin-3-(4H)-ylmethyl phosphoro dithioate from emergence of the plants until after all bolls were beyond the stage susceptible to insect attack.

The cotton varieties grown at St. Joseph were as follows: Coker 100 WR in 1956; Stardel in 1958; and Stoneville 3202 in 1959. DPL 15 was grown at Winnsboro in 1959. The defloration treatments were replicated four times in randomized block design. Individual plots consisted of three rows each 25 feet long.

Insecticide Experiment.—Treatments used in determining the effects of insecticides and schedules of applications on fiber quality were: none; 19 applications of toxaphene-DDT (2-1 pounds per acre) for complete control of cotton insects throughout the growing season; 9 applications of toxaphene-DDT (2-1 pounds per acre) and 9 applications of Guthion-DDT (0.25–0.50 pound per acre) for cotton insect control after the boll weevil infestation reached 25% punctured squares. The variety Stoneville 3202 was used in this experiment.

Treatments were replicated four times in a randomized block design. Individual plots consisted of 12 rows each 150 feet long. Cultural practices were identical in all plots of the experiment. There were moderate to heavy infestations of thrips, *Frankliniella fusca* (Hinds); tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois); cotton fleahoppers, *Psallus seriatus* (Reuter); and boll weevils, *Anthonomus grandis* Boheman in all plots until control was initiated. Insects in the insecticide treatments were effectively controlled after applications were begun. Applications of insecticides to the treated plots were all discontinued at the same time.

Yields were harvested in three pickings from the early and mid-season fruiting plots. Plots in the late fruiting treatment were harvested in two pickings. Cotton from plots treated with the various insecticides on different schedules of application was also harvested in two pickings. All the cotton from all pickings of each treatment was combined, blended and ginned on a roller-type gin. It was again blended after ginning and a composite sample removed. A 10-gram sample was removed from the composite for fiber testing.

Fiber analyses were made in the textile testing laboratory at Louisiana State University under controlled conditions of humidity and temperature. All testing was done after samples had been conditioned in the testing laboratory for 24 hours or longer. Fiber length was measured on a fibrograph. The upper-half mean length, as determined by use of the fibrograph, provides a measure of the average length expressed in terms of decimal fractions of an inch. Fiber fineness was measured by a micronaire. Micronaire readings indicate the resistance to passage of air through a 50-grain sample in a fixed chamber. Fiber strength tests were made using a ⅛-inch spacer between Pressley jaws (⅛-inch gauge), and a stelometer—a pendulum type fiber-strength tester.

RESULTS AND DISCUSSION.—Results of the fiber quality study for the defloration experiments are given in table 1.

There were no differences in fiber quality of the cotton produced in the early- and mid-season plots of the defloration experiments. However, there was a tendency toward a higher-quality fiber in the cotton from plots deflorated 3 weeks after fruiting began. Quality of Stardel fiber

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[2] Instructor, Louisiana Agricultural Experiment Station, Department of Home Economics, Louisiana State University, Baton Rouge.

[3] Assistant Professor, Louisiana Agricultural Experiment Station, Department of Entomology, Louisiana State University, Baton Rouge.

[4] Assistant Professor, Louisiana Agricultural Experiment Station, Department of Home Economics, Louisiana State University, Baton Rouge.

[5] Cooperation of the Northeast Louisiana Agricultural Experiment Station is acknowledged.

Table 1.—Comparison of fiber properties of cotton allowed to set fruit after defloration for 0, 3, and 7 weeks.

Fiber Characters[a]	No. of Weeks Fruit Removed	Year, Location, and Variety of Cotton			
		1956	1958	1959	
		St. Joseph			Winnsboro
		(Coker 100 WR)	(Stardel)	(Stoneville 3202)	(DPL 15)
Length	0	1.10	1.14	1.05	1.05
	3	1.08	1.16	1.06	1.04
	7	1.11	1.10**	1.02	1.06
Strength	0	16.5	18.4	14.7	17.3
	3	19.3	18.2	14.3	17.0
	7	18.6	18.1	16.2**	17.6
Fineness	0	4.39	3.78	4.30	4.78
	3	4.22	3.70	4.16	5.26
	7	4.40	3.18*	3.56*	3.10**

[a] See text for details.
* Significant at 5% level.
** Significant at 1% level.

produced at St. Joseph in 1958 by plants that were deflorated for 7 weeks after fruiting began was infeiror in fiber length and fineness. In 1959 Stoneville 3202 at St. Joseph produced fiber that was inferior in strength and fineness when deflorated 7 weeks. In 1959 at Winnsboro DPL 15 yielded finer fiber when the plants were deflorated 7 weeks. Coker 100 WR in 1956 at St. Joseph exhibited no quality differences regardless of the number of weeks the fruit was removed. Increased fiber strength of Stoneville 3202 in 1959 at St. Joseph is considered to be an indication of finer fiber. Other varieties did not react in this manner.

There were no differences in fiber quality in any variety any year at either location where deflorations were made for 3 weeks after first fruiting or where fruiting was allowed to develop normally.

Table 2.—Effect of several insecticides and schedules of application on Stoneville 3202 fiber quality of cotton. St. Joseph, Louisiana, 1959.

Treatments	Dosage (lb./acre)	Length (u.h.m. in inches)	Strength (g. per tex)	Fineness (micronaire)	Elongation (%)
Untreated check	0	1.05	14.0	4.45*	8.6
Toxaphene-DDT 19 applications	2–1	1.05	14.0	4.28	8.2
Toxaphene-DDT 9 applications	2–1	1.06	14.0	4.33	8.4
Guthion-DDT 9 applications	0.25–0.5	1.06	14.6	4.37	8.6

* Significant at 5% level.

Results obtained from the insecticide test are shown in table 2. Finer fiber was produced in plots treated with insecticides regardless of the application schedule.

Plants in treatment 2 (treated with 19 applications of toxaphene-DDT to give protection from thrips, fleahoppers, lygus bugs, bollworms, and boll weevils) produced cotton of the same quality as plants that received nine applications of toxaphene-DDT or Guthion-DDT, beginning when the boll weevil infestation reached 25%, to control boll weevils and bollworms.

Although cotton in all treatments where insecticides were used yielded finer fiber, the length, strength and elongation were not affected.

Systemic Activity of Dimethoate Applied to Cotton Seeds[1]

D. A. LINDQUIST,[2] JOSEPH HACSKAYLO,[2] J. C. CLARK,[2] and T. B. DAVICH[2]
College Station, Texas

ABSTRACT

Dimethoate was not exceptionally effective as a systemic cottonseed treatment against the boll weevil (*Anthonomus grandis* Boheman) and cotton aphid (*Aphis gossypii* Glover). Applied as a seed treatment, dimethoate caused considerable reduction in seedling emergence.

Studies with P^{32}-labeled dimethoate applied as a cottonseed treatment indicated that the toxicant was most rapidly absorbed 1 to 3 days after planting. Dimethoate seed treatment did not reduce the total emergence of artificially deteriorated seed, but did reduce the rate of emergence somewhat. Seeds deteriorated for 2 days absorbed more dimethoate than seeds deteriorated for 0, 1, 3, or 4 days.

Dimethoate was found to be less toxic than phorate to boll weevil larvae and adults.

Dimethoate, an organophosphorus insecticide, has shown some promise against certain internal parasites of animals (Drummond 1959, Rogoff et al. 1960) and for control of certain insect species attacking plants (Steinhauer et al. 1959, Harding 1959). The metabolism of dimethoate in mammals (Dauterman et al. 1959, Kaplanis et al. 1959) and plants (Dauterman et al. 1960) has been investigated.

Various aspects of problems related to treatment of cotton seeds with systemic insecticides for control of early-season cotton pests have been investigated (Parencia et al. 1957, 1957a, Reynolds et al. 1957, Lindquist et al. 1961, Hacskaylo & Ranney 1961). Most of these reports deal with phorate since this insecticide has proved to be the most effective seed treatment against cotton pests. The present study was undertaken to establish the systemic activity of dimethoate as a cottonseed treatment and the insecticidal effectiveness of dimethoate against the boll weevil (*Anthonomus grandis* Boheman).

MATERIALS AND METHODS.—*Seed Treatment.*—Deltapine-15 variety of cotton seeds was used in these experiments. To determine the phytotoxicity of dimethoate as a seed treatment, two sources of reginned seeds and a source

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[2] Entomology Research Division, Agric. Res. Serv., U.S.D.A.
[3] Crops Research Division, Agric. Res. Serv., U. S. D.A.

of acid-delinted seeds were treated with various dosages of the insecticide. The toxicant was applied to the seeds as a 50% carbon dust with a few drops of a 4% methyl cellulose as a sticker. The reginned seeds were planted in sand and seedling emergence was used as a measure of dimethoate phytotoxicity. Fifty acid-delinted dimethoate-treated seeds were planted, 10 each in soil in 1-gallon cans, and the emerging seedlings used for phytotoxicity and insecticidal activity measurements.

The effectiveness of dimethoate seed treatment against adult boll weevils and cotton aphids (*Aphis gossypii* Glover) was determined. Ten to 15 weevils were caged over each of two cans of cotton seedlings 7, 14, and 28 days after planting. The weevils were allowed to feed continuously on these plants for 5 days, after which the mortality of the weevils was recorded. A similar procedure was followed for the aphid assay except that 25 aphids were placed on the seedlings in each of two cans and the mortality of the aphids was recorded 3 days later. The aphid assays were run weekly after the seeds were planted. Weevil and aphid assays were run on seedlings grown from untreated seeds for controls. Cotton seeds treated with 4 mg. of phorate per seed were used for comparison. All mortality data were corrected for control mortality by Abbott's formula (Abbott 1925).

P^{32}-labeled dimethoate[4] with a specific activity of about 4 millicuries per gram was used to estimate the absorption of dimethoate by treated cotton seeds. Doses of 1 and 4 mg. of the radiolabeled toxicant per seed were used. In order to have as uniform seeds as possible, double haploid No. 8984 Deltapine-15 variety of cotton seeds was used. Fifty seeds treated with each dosage were planted in sand in 1-gallon cans at the rate of five seeds per can. At intervals after planting the embryos or emerging seedlings were harvested. The seed coats were removed from the embryos; the embryos then were rinsed with water, air-dried, and radioassayed. The entire seedling, including the roots, was harvested, dried at 60° to 70° C., ground to pass a 40-mesh screen, and 100 to 200 mg. aliquots radioassayed. All radioassay measurements were made with a thin-end window Geiger-Muller tube and a conventional scaling unit.

To determine the effect of dimethoate on deteriorated seeds, groups of seeds were artificially deteriorated by exposing them to a saturated atmosphere at 50° C. for 0, 1, 2, 3, or 4 days as described by Presley (1958). Twenty-five seeds from each deterioration time then were treated with 2 mg. of P^{32}-labeled dimethoate per seed as described above. This dosage was chosen because preliminary tests had shown that a dose of 4 mg. per seed often was phytotoxic, causing drastic reduction in emergence and increased leaf shed. These treated seeds were planted in sand in 1-gallon cans, five seeds per can, and the seedling emergence recorded. Seedlings were harvested 7, 9, 16, and 23 days after planting for radioassay measurements. Twenty-five untreated seeds from each deterioration time were planted as controls.

Insecticidal Activity.—The insecticidal activity of dimethoate against boll weevil larvae and adults was investigated. Phorate was included for comparison. Tests against the larvae were conducted by adding the insecticide to an artificial diet used for rearing boll weevils in the laboratory (Brazzel et al. 1959). The toxicants, as ethanol solutions, were incorporated into the diet to make final concentrations of 0.1, 1.0, and 10.0 p.p.m. Each dosage was tested in four petri dishes containing a total of 196 eggs. An equal number of untreated dishes was used as controls. Egg hatch and adult emergence were recorded with the reduction of the latter as the criterion of toxicity.

Table 1.—Effectiveness of dimethoate cottonseed treatments against adult boll weevils and emergence of cotton seedlings from treated seeds.

Compound	Dosage (mg./seed)	Per Cent Seedling Emergence After 8 Days			Five-Day Weevil Mortality at Indicated Days After Planting[a] (%)		
		1[b]	2[c]	3[d]	7	14	28
Control	—	78	84	96	0	0	0
Dimethoate	1	50	51	70	91	0	—
	2	37	38	86	100	26	7
	4	19	18	82	100	51	6
Phorate[e]	4	48	59	88	100	86	54

[a] Average of 1 to 4 tests of 20 to 30 weevils per test.
[b] Source 1—reginned seeds, 200 seeds per dose, planted in sand.
[c] Source 2—reginned seeds, 200 seeds per dose planted in sand.
[d] Source 3—acid-delinted seeds, 50 seeds per dose, planted in soil. Boll weevil tests run on these plants.
[e] Included for comparison.

The toxicity of dimethoate, the oxygen analogue of dimethoate (*O,O*-dimethyl *S*-(N-methylcarbamoylmethyl) phosphorothiolate), and phorate to adult 3- to 5-day-old weevils was determined by topical application. The weevils were anesthetized with carbon dioxide and treated on the dorsal prothoracic region with 1 microliter of acetone containing the toxicant. The treated weevils were fed cotton seedlings and held at 85° F. and 40% to 60% relative humidity. Mortality was recorded 48 hours after treatment. Three to five groups of 20 to 30 weevils each were used for each dosage.

RESULTS.—*Seed Treatment.*—The variation in seedling emergence between sources of cotton seed (table 1) undoubtedly was caused by variations in seed quality. Seedling emergence was reduced by all dimethoate treatments but was not always correlated with the dosage. Dimethoate was more phytotoxic than phorate under the test conditions used.

The insect assay data, based on the plants grown from acid-delinted seeds planted in soil, showed that dosages of 1, 2, and 4 mg. of dimethoate per seed resulted in 91%, 100%, and 100% mortality, respectively, of the test boll weevils at 1 week after planting, and 0%, 26%, and 51% mortality, respectively, at 2 weeks. Dosages of 4 mg. of phorate per seed resulted in 100%, 86%, and 54% mortality at 1, 2, and 4 weeks, respectively, after planting. Four milligrams of dimethoate per seed gave complete kill of the cotton aphid for 5 weeks. Comparable tests with phorate resulted in complete kill of aphids for 6 weeks.

The absorption of P^{32}-labeled dimethoate by cotton seeds is shown in table 2. The data from the undeteriorated seeds treated with 2 mg. of dimethoate per seed are included for comparison. These data show that plants grown from seeds treated with 1 or 2 mg. of dimethoate per seed absorbed nearly the same amount of dimethoate. Those treated with 4 mg. of dimethoate per seed absorbed about twice the amount of toxicant as plants grown from

[4] Obtained from Volk Radiochemical Co., 5412 N. Clark St., Chicago 40, Ill.

Table 2.—Absorption of P^{32}-labeled dimethoate by cotton seedlings following seed treatment.[a]

Days After Planting	Dimethoate Equivalents per Plant (μg.) at Indicated Dosage per Seed		
	4 mg.	2 mg.	1 mg.
1	22	—	10
3	111	—	50
4	133	—	52
7	—	50	—
15	167	—	87
16	—	78	—
21	195	—	87
23	—	58	—
28	243	—	72
35	146	—	83

[a] Average of 3 to 5 plants.

seeds treated with 1 or 2 mg. of dimethoate. The data in this table also show that the most rapid dimethoate absorption occurred between 1 and 3 days after planting. This is similar to the absorption of phorate by cotton seeds (Lindquist et al. 1961).

The emergence of the treated and untreated artificially deteriorated cotton seeds is shown in table 3. The 3- and 4-day deterioration times reduced the rate of and total emergence over the 0-, 1-, and 2-day deterioration times. However, the dimethoate treatment did not cause a significant reduction in total emergence of the deteriorated seeds, but did reduce the rate of emergence somewhat.

Insecticidal Activity.—Dosages of 0.1, 1.0, and 10.0 p.p.m. of dimethoate in the larval diet resulted in mortalities of 5%, 12%, and 100%, respectively. Equivalent doses of phorate gave 0%, 100%, and 100% mortality, respectively. Egg hatch was not affected by either toxicant in this study.

The dosage-mortality curves obtained by topical application of dimethoate, the oxygen analogue of dimethoate, and phorate resulted in LD-50 values of 1.5, 2.2, and 0.7 micrograms of toxicant per weevil, respectively (fig. 1). These results show that the oxygen analogue of dimethoate was not so toxic to boll weevils as the parent compound when applied topically. The oxygen analogue has been reported to have more antiesterase activity than the parent compound (Anonymous 1959). Paper chromatography (Hacskaylo et al. unpublished data) showed that this material was 90% to 95% pure.

Table 3.—Emergence of artificially deteriorated cotton seeds with and without dimethoate seed treatment (2 mg. per seed).[a]

Deterioration Time (days)	Emergence at Indicated Days After Planting (%)									
	Treated					Untreated				
	4	5	6	7	8	4	5	6	7	8
0	80	92	92	92	92	96	96	96	96	96
1	60	88	88	88	88	88	88	88	88	88
2	8	80	88	88	92	96	100	100	100	100
3	0	48	64	64	64	52	68	68	68	72
4	12	20	36	52	52	12	28	40	52	52

[a] Twenty-five seeds used for each deterioration time.

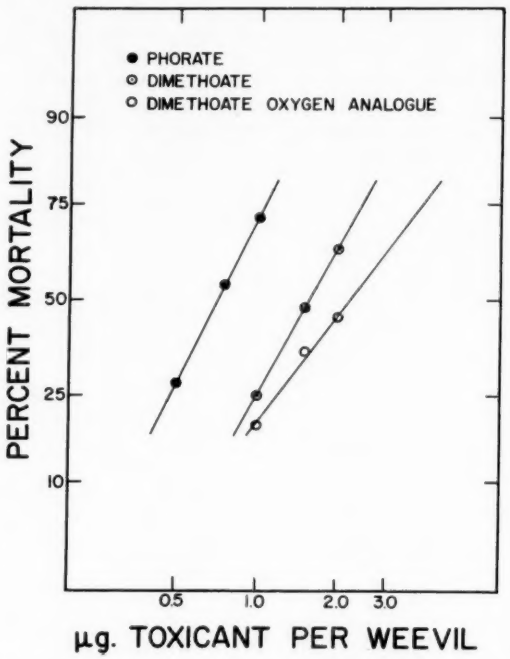

FIG. 1.—Toxicity of dimethoate, dimethoate oxygen analogue, and phorate applied topically to adult boll weevils.

DISCUSSION.—The data presented show that dimethoate is less effective than phorate against boll weevils and cotton aphids when these materials are applied as seed treatments. This difference in effectiveness is not a result of different amounts of each toxicant absorbed; the amounts of dimethoate absorbed compare closely with the amounts of phorate absorbed following seed treatments (Lindquist et al. 1961). This finding was not expected because dimethoate is considerably more water-soluble than phorate. Dimethoate was found to be soluble to about 15,000 p.p.m. (1.5%) in water at 30° C. Phorate is soluble to about 50 p.p.m. in water (Anonymous 1955). Since the difference in water solubility did not affect absorption markedly, other factors must be involved.

The variations in seedling emergence of different sources of cotton seed treated with the same dose of dimethoate are undoubtedly reflections of seed quality. The reginned seeds (sources 1 and 2, table 1) were obviously of poorer quality than the acid-delinted seeds (source 3, table 1). It is apparent from these limited tests that seed quality, as reflected in seed viability, is important not only in testing candidate systemic insecticides but also in their use by the farmer. Breaks in the seed coat, often found in reginned seed, probably increase the chances of insecticidal seed treatments, reducing emergence. Previous work by Hacskaylo & Ranney (1961) has shown that reductions in stand caused by phorate seed treatment were influenced greatly by seed quality.

More dimethoate was accumulated by plants grown from the 2-day deteriorated seeds than any of the other treatments (table 4). Also, the percentage of emergence

Table 4.—Dimethoate-equivalents found in cotton plants following dimethoate seed treatment (2 mg. per seed).[a]

Deterioration Time (days)	Dimethoate-Equivalents (μg./plant) at Indicated Days After Planting			
	7	9	16	23
0	50	70	78	58
1	57	52	72	101
2	110	116	93	130
3	33	71	78	72
4	54	41	55	90

[a] Average of 2 to 5 plants.

of these seeds was as high as or higher than the controls. This higher emergence indicated that the 2-day deterioration period caused some change in the cotton seed that did not reduce total emergence but did result in a more favorable condition for dimethoate absorption. This change could have been a slight rupturing of the seed coat or it might have been an increased permeability of the cell membranes reflected by increased dimethoate absorption. In either event, the change in the seeds resulting in more dimethoate absorption probably was brought about by the extent of germination after 2 days of exposure to the conditions used for seed deterioration.

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The Effect of Wing Movement by the Green Peach Aphid on the Transmission of Lettuce Mosaic Virus[1]

Donald L. McLean,[2] *University of California, Davis*

Abstract

Investigations were conducted to determine if wing movement of alate green peach aphids, *Myzus persicae* (Sulzer), would affect transmission efficiency of lettuce mosaic virus when compared with transmission efficiency of alates not allowed wing movement and apterous adults. All aphids tested were attached to pins with watercolor paint. Single aphids were used in 30 tests with each of the 3 groups. Treatment times used for the groups were 0, 15, 30, 60, 900, 1,800, and 2,700 seconds. Preceding treatment, all aphids were given a 15-second artificially interrupted access feeding on a lettuce mosaic virus source plant.

The results indicated that regardless of form, transmission efficiency was similar at any time period. Loss of transmission efficiency was similar for all groups.

Studies conducted with vector-virus relationships of aphid-borne nonpersistent viruses have been primarily concerned with the use of apterous aphids as vectors. Sylvester (1955) observed that alate green peach aphids (*Myzus persicae* (Sulzer)) were less efficient vectors of lettuce mosaic virus than either the adult apterae or nymphs.

The investigations described in this paper were conducted to ascertain if wing movement of alate green

[1] Accepted for publication May 19, 1961.

[2] Assistant Entomologist, Department of Entomology and Parasitology, University of California, Davis.

peach aphids would affect, or in any way influence, aphid transmission efficiency of lettuce mosaic virus.

METHODS.—Several last instar alatoid green peach aphid nymphs were removed from colonies on smooth leaf mustard, *Brassica juncea* Cos., and placed on caged Great Lakes variety lettuce plants. This procedure was carried on each day to insure a constant supply of alates. A similar procedure was employed with apterous forms using separate cages. Although the majority of the alate and apterous nymphs remained on the lettuce plants, many of the adults migrated to the sides of the cage and remained there. Whether this was due to a reluctance to accept lettuce as a food plant or positive phototropism is not known. Only those aphids that were on the sides of the cage were chosen for testing.

The exact adult emergence time for both aphid forms was not determined. However, upon completion of tests each day, all remaining adult aphids were removed, so that those used the following day were adults for less than 24 hours.

The original lettuce mosaic virus source plant was collected from a field of commercially cultivated Great Lakes variety lettuce, *Lactuca sativa* L., in the area of Salinas, California. The virus source used was variant I of lettuce mosaic virus (McLean & Kinsey 1961). Source and test plants were Great Lakes variety lettuce with the test plants chosen at random from a test population and only plants with one primary leaf were used. The virus source plants were chosen from plants infected in a previous experiment.

For the purpose of testing, 3 groups of aphids were used: 2 groups of alates (1 allowed wing movement, the other not), and 1 of apterous adults.

Number 0 insect pins were pushed through number 6 corks so that the heads of the pins were flush with the sides of the corks. The protruding ends of the pins were bent down at slightly less than a right angle about $\frac{1}{2}$ inch from the cork. The fine tips of the pins were then bent up at slightly more than a right angle to the first bend. The aphids were attached to the ventral part of this tip and by using the corks as supports for the pins, movement of the attached aphids was facilitated (fig. 1). Both

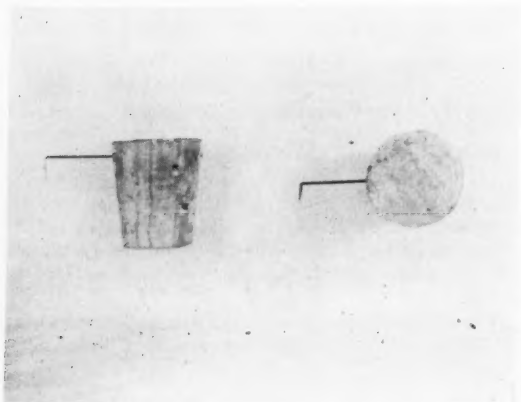

FIG. 1.—The shape of a number 0 insect pin to which the aphids are attached and the number 6 cork used as the base for the pin.

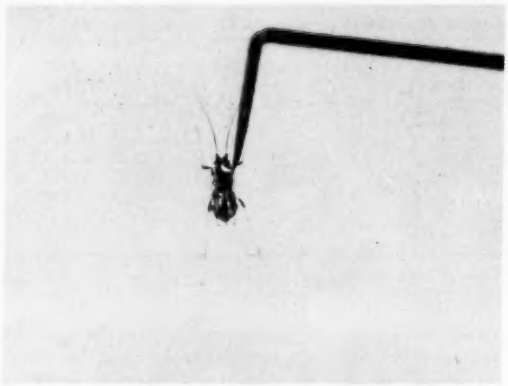

FIG. 2.—Wing movement by an alate green peach aphid and point of attachment to the pin.

aphid forms were attached in the prothoracic region under a microscope. White watercolor paint[3] was used as the adhesive to fasten the aphids to the pins (Johnson 1958). This material was nontoxic to the aphids and would set very quickly. Removal of the aphids from the pins was accomplished by merely touching the aphid on the thorax with a camel's-hair brush.

A glass tube was drawn out to a fine tip so that by aspiration it would accept only the wings of the alates, which resulted in easy manipulation of the aphids (Johnson 1958). The apterous forms were manipulated with a camel's-hair brush. Alates not allowed wing movement were placed on the pins and before wing movement occurred the wings were fastened together with a small dab of watercolor paint. The other group of alates was allowed free wing movement (fig. 2).

Time periods used for testing were 0, 15, 30, 60, 900, 1,800, and 2,700 seconds. Each completion of the time series with the three aphid groups constituted a test and was completed before another series was undertaken. Single aphids of each form were used for each treatment time tested. The times to be tested and the aphid forms tested were chosen at random within each test with 30 tests conducted with each of the three groups. Each aphid following removal from the cage was allowed a 15-second artificially interrupted feeding on the source plant before being placed on the pin.

Following the fastening of the aphids on the pin, a mean time before initiation of wing movement was found to be 20 seconds and this was subsequently taken to be the time limit. Initiation of wing movement of the alates was often difficult to obtain during this period and if wing movement did not occur, the aphids were discarded. A quick movement of the cork would many times initiate wing movement. Additionally, those alates not allowed wing movement and the apterous adults were on the pin 20 seconds before test timing began. The 15-, 30-, and 60-second timing was done by using a stop watch and the longer periods by using an electric wall clock. Following fastening of the aphids on the pins, the corks were placed next to a window and the aphids observed closely. If any interruption of wing movement occurred before time was

[3]Chinese White manufactured by M. Grumbacher, New York, New York.

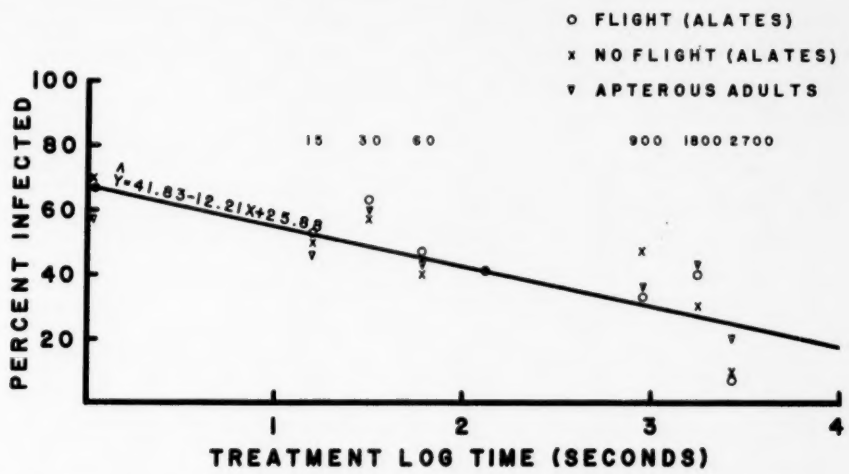

Fig. 3.—Regression line indicating loss of transmission efficiency of lettuce mosaic virus by the three aphid groups. Flight refers to wing movement when aphid was attached to a pin, and not free flight.

Table 1.—Results of tests conducted to determine if wing movement of alate green peach aphids would affect transmission efficiency of lettuce mosaic virus.

Treatment Time (seconds)	Per Cent Infected: Wing Movement (Alates)	No Wing Movement (Alates)	Apterous Adults	X^2
0	—	70.0	56.7	0.50
15	53.3	50.0	46.7	≈0.94
30	63.3	56.7	56.7	≈0.94
60	46.7	40.0	40.0	0.90
900	33.3	46.7	33.3	≈0.60
1,800	40.0	30.0	40.0	≈0.73
2,700	6.7	6.7	20.0	0.15

up, the aphid was discarded. Halting wing movement at the end of the desired time was effectively accomplished by blowing on the aphid's dorsum.

Following treatment, the aphids were placed individually in plastic cages and left on the test plants for 1 hour after which they were removed and the plants fumigated.

Results.—The results indicate that regardless of form, the aphid groups did not show significant variation in transmission efficiency from one another at any given time period (table 1). Even with increased metabolic activity in the alate aphids that were allowed wing movement transmission efficiency was little affected.

A regression line was calculated from the data ($Y = 41.83 - 12.21\ X + 25.88$) and indicates a similar loss of transmission efficiency for all groups (fig. 3). At the 2,700-second point, efficiency dropped rapidly and whether this is due to loss of virus by the vector or physiological exhaustion of the aphids is not known. Although not significantly different from one another ($X^2 = 3.23$, $df = 2$, $p = 0.15$), the 2,700-second points do indicate a higher transmission efficiency by the apterous forms. In like regard, although also not significant, the 0 second points indicate a higher transmission efficiency by the alates not allowed wing movement over the apterous forms ($X^2 = 0.42$, $df = 1$, $p = 0.50$).

Since transmission efficiency only varied slightly regardless of aphid form, it would tend to support mechanical transmission of nonpersistent viruses. If the virus were other than a contaminant on the stylets it would seem reasonable that the physiological stress of flight might alter the insect's capacity to transmit if the virus were taken internally. In any case these data are not opposed to the mechanical transmission of nonpersistent viruses.

Additionally, it is interesting to note from the data that an alate green peach aphid allowed wing movement still has the ability to transmit lettuce mosaic virus at 2,700 seconds. Consequently, it may not be unreasonable to assume that free-flying viruliferous aphids could fly for some distance and still have the ability to cause infection. If, on the other hand, the viruliferous alates were transported by wind, possibly more distance could be covered before losing the virus charge. In an area such as the Salinas Valley of California, where lettuce production is concentrated during most of the year, viruliferous green peach aphids might cause infection centers over a large area from which the virus could spread.

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Feeding and Oviposition Habits of the Jack-Pine Budworm[1]

HERBERT M. KULMAN and ALEXANDER C. HODSON[2]

ABSTRACT

The jack-pine budworm, *Choristoneura pinus* Freeman, bored into the main axis of staminate cones, cone-bearing twigs and scored the surface of twigs. On nonstaminate cone-producing trees the budworm scored the surface of twigs, bored into twigs and mined the fascicles of new needles. Larvae occasionally fed on the sides of old needles. The budworm was not observed mining new needles. Larvae in marquisette cages consumed about 50 needles per larva, but the feeding habits differed from those of uncaged larvae.

Seventy-eight per cent of the egg masses of caged adults were laid in two rows on the underside of needles.

In the spring of 1957 and 1958 observations were made on the feeding habits of the jack-pine budworm, *Choristoneura pinus* Freeman, as a part of a larger study on the nature and effects of defoliation by the budworm on jack pine. The following material, although not complete, is being published because the authors will not have an opportunity to continue the study in the near future.

LARVAL FEEDING HABITS.—In spring the second-instar larvae on staminate cone-bearing trees were observed feeding in the staminate cones and eating deep holes in the central axis of the staminate cones and in the surface of the cone-bearing portion of the branch. A few larvae were found boring in the center of the cone-bearing twigs. The staminate cones and the cone-bearing branch tips were so riddled that they appeared to be held intact only by the silk webbing made by the budworms. Some larvae apparently live in the staminate cones until pupation, but it more often appeared that the staminate cone cluster served only as a retreat tunnel site and that the older larvae fed on nearby foliage. In a few cases staminate cones contained large active budworms and the nearby needles showed no sign of damage. Some larvae were observed in the tunnels eating needles that were chewed off and pulled into the tunnel. On many staminate cone-producing tips current growth was not produced and the larvae were feeding on old foliage. Under these circumstances it was not unusual to find needles only eaten along the sides as shown in figure 1.

The feeding habits of the budworm on nonstaminate cone-producing trees were also quite varied. In spring the second-instar larvae were observed feeding on new needles and feeding on the sides of elongating shoots, especially where two or more were close together. They were also found boring into the fascicles of new needles and eating the needles off at the base inside the fascicle as shown in figure 2. A few larvae were found mining the elongating shoots. Budworms were never observed mining new needles as stated by Graham (1935) or old needles as stated by Shenefelt & Benjamin (1955). MacAloney & Drooz (1956) state that the jack-pine budworm does not mine needles. No references were found concerning the fascicle- or stem-mining habit. The habit of feeding on the sides of elongating shoots and of webbing the shoots together was very prevalent. Some shoots were more than 50% girdled and the feeding scars were often 4 inches long, 0.5 inches wide, and up to 0.1 inch deep. Several buds on heavily infested trees were mined, but at no time were insects found in the mines. The nature and effect of budworm feeding on pistillate cones will be covered as a part of an article now in preparation.

When several elongating shoots occur on one tip the

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[2] Assistant Professor of Entomology, West Virginia University and Professor of Entomology and Economic Zoology, University of Minnesota, respectively.

FIG. 1.—Old needles partly eaten by early instar jack-pine budworm larvae.

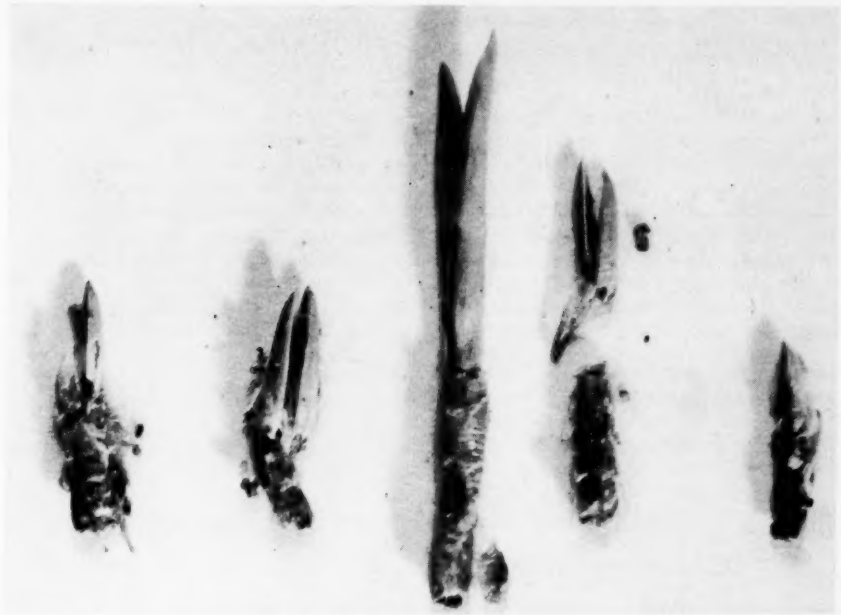

FIG. 2.—Fascicle mining of new needles by early instar jack-pine budworm larvae.

larvae web them together and as the tips elongate they twist and buckle.

Larvae that are disturbed retreat quickly into the web shelter. If they are knocked off the branch they are usually connected to the branch by a strand of silk. If they do not come in contact with another branch the larvae will pull themselves back up by catching the strand of silk with their mouth parts and roll the silk into a ball between their thoracic legs.

In order to determine the number of needles eaten by a jack-pine budworm larva, individual larvae (mostly third and early fourth instars) were caged on insect-free branches of jack, Scotch, red, and white pines. The larvae were caged on the same host species from which they were collected. The cages were 6 inches wide and 10 inches long and made of nylon marquisette. Parasitism and unknown causes of death disqualified 18 of the 38 cages. The caged branches were collected in late July and counts were made of the number of missing needles. These data are tabulated in table 1.

Without exception the entire needle was consumed rather than eaten off at the base and webbed to the tree, which is the accepted normal feeding habit of the budworm. The web tunnels, which are normally associated with the partly eaten needles, were also absent. Because of these striking differences these data do not represent the feeding activities as they normally occur in the field. No conclusions are possible at this point, but it can be implied that the larvae were not stimulated to construct web retreats and feeding shelters because the cage afforded sufficient protection. It may further be suggested that the construction of a web tunnel is a function of need or at best a habit triggered only by specific conditions, and that the habit of eating only part of the needle may be related to the shelter-building habit of the budworm and not necessarily a specific feeding habit. Certain weather conditions, especially overcast conditions, that afford the same type of stimulus (or lack of stimulus) as the cages, may have an effect on the number of needles destroyed. The possibilities become increasingly interesting in light of Wellington's work (1952) on the spruce budworm in which he stated that, "Ideal physical conditions for spruce budworm development occur when the air is relatively dry and clear."

Table 1.—Needle consumption of caged jack-pine budworm larvae on four species of pine.

SPECIES OF PINE	AVERAGE NUMBER OF NEEDLES CONSUMED			NO. OF OBSERVATIONS
	New	Old	Total	
Jack	48.4	1.5	49.9	14
Scotch	41.0	5.5	46.5	2
White	27.5	5.0	32.5	2
Red	30.5	0.0	30.5	2

OVIPOSITION HABITS.—Campbell (1953) states that the jack-pine budworms deposit their eggs in two rows and the spruce budworm in three rows. Shenefelt & Benjamin (1955) state that the eggs of the jack-pine budworm are deposited in three rows on the underside of needles.

In late July of 1958 about 200 pupae were collected and placed in a large cage with several jack pine branches. After the adults emerged, mated, and laid eggs, the egg masses were examined. Results of these findings are shown in table 2. From these data it appears that the jack-pine budworm usually oviposits in two overlapping rows on the concave surface of the needle. It was noted that when eggs were deposited on the convex surface of

Table 2.—Location and number of rows of eggs deposited by the jack-pine budworm.

Number of Rows of Eggs	On Concave Surface of Needle	On Convex Surface of Needle
2	92	0
3	9	11
4	0	6

the needles that the number of rows appeared to be determined by the width of the surface. Therefore, it might be suggested that the number of rows of eggs is determined more by the width of the oviposition site than by specific oviposition habits.

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Distribution and Solubility Properties of Phosphoric and *O, O*-Diethyl Phosphorodithioic Acids Fed to Laying Hens[1]

H. W. Dorough and B. W. Arthur, *Department of Zoology-Entomology, Agricultural Experiment Station, Auburn, Alabama*

Abstract

Phosphorus^{32}-labeled phosphoric acid and *O,O*-diethyl phosphorodithioic acid were fed at 100 p.p.m. in the diet of laying hens for 7 days. The liver, kidney, and bone accumulated more radioactive materials than the blood, brain, breast, fat, feathers, gizzard, or skin. Egg yolk contained more acid equivalents than the shell or white. Acetonitrile-soluble residues were detectable in the feces, egg yolk, and liver. About 46% of the phosphoric acid and 36% of the diethyl phosphorodithioic acid consumed in the feed were present in the feces 14 days after treatment of hens. The phosphoric acid and diethyl phosphorodithioic acid exhibited solubility properties characteristic of many organophosphate insecticides.

Where specific analytical methods have not been developed, radioisotopes are often used in the quantitative determination of organophosphate residues in animal and plant tissues. Residue data based upon the selective solvent extraction of the radiolabeled insecticide from an aqueous macerate of the biological media into an organic solvent may be misleading. Good recoveries of Co-Ral® (*O,O*-diethyl *O*-3-chloro-4-methyl-2-oxo-2H-1-benzopyran-7-yl phosphorothioate) were reported from poultry tissues using acetone and benzene as primary extraction solvents followed by *n*-hexane and acetonitrile partitioning as a cleanup procedure (Dorough et al. 1961a). Above 80% of the P^{32}-Co-Ral added to tissues of laying hens was recovered in the acetonitrile fraction. However, in the analyses of tissues and eggs from hens treated with Co-Ral (Dorough et al. 1961a) or Ruelene®(*O*-4-*tert*-butyl-2-chlorophenyl *O*-methyl methylphosphoramidate) (Buttram & Arthur 1961), the possibility exists that the organic solvent soluble P^{32} materials represented the parent compounds and degradation products that had been incorporated into normal phosphorus-containing metabolites.

Most extraction procedures used in residue determinations leave from 50% to 80% of the P^{32} materials in the tissue residue. These P^{32} materials are usually classified as unextractables and are considered nontoxic. The unextractable Co-Ral equivalents in the liver and feces of hens dusted with Co-Ral or hens receiving Ruelene in the feed were fractionated into acid-soluble compounds, phospholipids, ribonucleic acid, and desoxyribonucleic acids (Dorough et al. 1961a, Buttram & Arthur 1961). Since these insecticides were degraded to phosphoric acid and incorporated into naturally occurring phosphorus-containing metabolites, the possibility that P^{32} phosphoric acid and *O,O*-diethyl phosphorodithioic acid would follow the same distribution and solubility patterns was investigated.

Procedure.—*Materials.*—Ten mc. of P^{32}-labeled phosphoric acid was converted to phosphorus^{32} pentasulfide according to the isotope exchange method of Casida (1958). Based on the theoretical yield of phosphorus pentasulfide, 4 molar equivalents of dry ethyl alcohol and 3 ml. of toluene were introduced into the reaction flask and the mixture was refluxed for 3 hours to yield the *O,O*-diethyl phosphorodithioic acid (March et al. 1956). The free acid was converted to the more stable salt by adding equimolar potassium hydroxide. The crude potassium salt of the diethyl phosphorodithioic acid was crystallized twice from benzene and was purified further on an anion exchange column (Plapp & Casida 1958). The product was 85% pure as determined by its chromatographic behavior on the anion exchange column, and the specific activity of the final column purified product was 660 c.p.m./μg.

Ten mc. of radiolabeled phosphoric acid was used as

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received from the Atomic Energy Commission with the exception of removing the HCl carrier by evaporation.

Treating and Sacrificing Hens.—Twenty White Leghorn hens were housed singly in wire cages equipped with dropping boards. Eight of the hens were fed P^{32} phosphoric acid mixed in the feed at 100 p.p.m. for 7 days and another eight hens received the diethyl phosphorodithioic acid at the same concentration for the same period. The remaining four hens served as untreated controls.

Each of the P^{32}-labeled phosphoric acids was mixed with 8 pounds of laying mash. The feed was mixed until homogeneous as determined by radioassay of several 25-mg. samples. Water and P^{32} feed were provided the hens at all times. The feed hoppers were secured to the outside of the cages to prevent contamination of the feces with the waste material.

Records were kept on the amount of feed consumed daily by each hen. The average weight of hens fed P^{32} phosphoric acid was approximately 1.7 kg., and the average weight of the hens receiving diethyl phosphorodithioic acid was 1.8 kg. Egg production was about 75% throughout the experimental period. The eight hens receiving each of the P^{32} phosphoric acids were sacrificed in groups of 2 at 1, 3, 7, and 14 days after initiating the experiment. At the end of the 7-day feeding of the P^{32} mash, four of the hens were held on untreated feed for an additional 7 days (referred to in the tables as the 14-day time interval). The following tissues were taken from each hen for analysis: blood, brain, bone (thigh), breast, drumstick, fat, feathers, gizzard, kidney, liver, and skin (back). Ten ml. of blood was withdrawn from the heart region with a 10 cc. syringe prior to sacrificing the hens. The blood was mixed with 2.0 ml. of 1% heparin and stored in a refrigerator until analyzed. All other tissues were wrapped separately in aluminum foil and stored in deep freeze until analyzed.

The four untreated hens were used to determine the efficiency of the extraction procedures in removing known amounts of added phosphoric acid and diethyl phosphorodithioic acid from the tissues.

Analytical Methods.—Tissues, eggs, and feces were analyzed for total P^{32} materials according to methods of Dorough et al. (1961a). The liver, egg yolk, and feces were also subjected to the fractionation procedure described for removing Co-Ral and Ruelene from poultry tissues (Dorough et al. 1961a, Buttram & Arthur 1961). This fractionation procedure consisted of acetone followed by benzene extraction of the macerated tissues, partitioning the residue of the acetone-benzene extracts between *n*-hexane and water, and finally, partitioning the *n*-hexane solubles with an equal volume of acetonitrile. About 20 g. of liver from each hen, 20 g. of egg yolk taken from eggs laid on the day the hens were sacrificed, and 20 g. of a composite sample of feces were fractionated. The radioactivity in the acetonitrile, *n*-hexane, water, and tissue residue was determined.

The acetonitrile-soluble radioactivity of the liver and egg yolk was chromatographed on a Celite partition column, using acetonitrile saturated with a 1:1 mixture of *n*-heptane and cyclohexane as the stationary phase and a 1:1 mixture of *n*-heptane and cyclohexane saturated with acetonitrile with 5% chloroform (v/v) added to the final volume as the mobile solvent (Vickery & Arthur 1960). The acetonitrile-soluble materials of the feces were too low in activity for Celite chromatography, but the water-soluble materials were chromatographed on an anion exchange column (Plapp & Casida 1958).

The radioactivity remaining in the feces, liver, and egg yolk residue after the acetone and benzene extraction was separated further into acid-soluble compounds, phospholipids, ribonucleic acid, and desoxyribonucleic acid (Schneider 1945).

RESULTS AND DISCUSSION.—*Residues in Tissues and Eggs.*—The average feed consumption was 87 g. per hen per day and corresponded to a daily intake of 3.98 mg. of either acid per kg. of body weight. The total P^{32} phosphoric and *O,O*-diethyl phosphorodithioic acid equivalent in tissues are given in table 1. The P^{32} materials were concentrated in the liver, bone, and kidney to a greater extent than in other tissues. In most instances, the total p.p.m. of P^{32} residues increased proportionately with the time the hens were on radioactive feed. When the hens were returned to normal feed for 1 week, the P^{32} residues decreased in all tissues except the brain, breast, drumstick, and skin. These tissues actually contained slightly more P^{32} residues after 14 days than after 7 days (table 1). The radioactivity in such metabolically active organs as the liver and kidney declined rapidly when the hens were returned to normal feed.

The P^{32} residues in tissues of hens following treatment with phosphoric acid or its diethyl ester derivative were about the same as observed in tissues following treatment of hens with Co-Ral (Dorough et al. 1961b) or Ruelene (Buttram & Arthur 1961).

The p.p.m. of P^{32} phosphoric acid and diethyl phosphorodithioic acid equivalents in the white, yolk, and shell of eggs are shown in table 2. The radioactivity in egg yolk was considerably higher than in the white or shell of eggs following treatment of hens with phosphoric acid. The highest concentration of phosphoric acid equivalents in the yolk was at 1 to 3 days after the hens were returned to normal feed (8 to 10 days after initiating the experiment). There were no detectable radioactive materials in the white of eggs from hens receiving diethyl phosphorodithioic acid in the feed for 7 days. The highest concentration of this dithioic acid appeared in the yolk after the hens were on treated feed for 7 days. In both tissues and eggs, P^{32} phosphoric acid accumulated to a

Table 1.—Phosphoric and diethyl phosphorodithioic acid equivalents in tissues of laying hens fed radioactive feed.[a]

	P.P.M. AT INDICATED DAYS AFTER TREATMENT[b]							
	Phosphoric Acid				Diethyl Phosphorodithioic Acid			
TISSUE	1	3	7	14	1	3	7	14
Blood	0.10	0.08	0.33	0.25	0.14	0.33	0.19	0.38
Bone	.22	.80	1.69	.81	.57	1.28	3.09	.81
Brain	.01	.07	.22	.27	.48	.09	.19	.24
Breast	.07	.16	.49	.52	.14	.19	.38	.71
Drumstick	.09	.17	.40	.54	.09	.28	.43	.71
Fat	.06	.05	.13	.03	.19	.19	<.01	<.01
Feathers	.02	.02	.05	.03	.66	.09	<.01	<.01
Gizzard	.20	.44	.75	.52	.08	.41	.16	.08
Kidney	.55	.88	1.42	.64	.86	1.33	1.05	.76
Liver	1.02	1.26	2.17	.78	1.05	1.76	1.81	.62
Skin	.04	.10	.25	.12	.09	.19	.09	.38

[a] Phosphoric acids mixed in feed at 100 p.p.m. and fed to hens for a maximum of 7 days.

[b] Averaged from two hens sacrificed at each time interval with duplicate analyses per tissue.

Table 2.—P.p.m. phosphoric acid and diethyl phosphorodithioic acid equivalents in eggs from hens receiving radioactive feed.[a]

Days After Treatment	Phosphoric Acid[b]			Diethyl Phosphorodithioic Acid[b]		
	White	Yolk	Shell	White	Yolk	Shell
1	<0.01	<0.01	0.03	<0.01	<0.01	0.07
2	.03	.78	.43	< .01	< .01	.16
3	.04	2.66	.86	< .01	< .01	.27
5	.09	1.73	.88	< .01	.13	.14
7	.11	2.53	1.19	< .01	1.26	.24
9	.12	3.58	.52	< .01	1.13	.12
11	.05	3.04	.48	< .01	1.08	.03
13	.04	2.43	.12	—	—	—

[a] Phosphoric acids mixed in feed at 100 p.p.m. and fed to hens for a maximum of 7 days.
[b] Averaged from 2 to 10 analyses at each time interval.

greater extent than the diethyl phosphorodithioic acid.

Fractionation of Eggs, Liver, and Feces.—Some P^{32} phosphoric and diethyl phosphorodithioic acid equivalents partitioned from water into *n*-hexane and, finally, into acetonitrile (table 3). As high as 0.2 p.p.m. of acetonitrile-soluble radioactive materials was present in yolk of eggs from hens fed 100 p.p.m. of P^{32} phosphoric acid in the diet. The liver also contained some acetonitrile-soluble P^{32} materials but the feces contained considerably less. Since very little P^{32} phosphoric acid appeared in the acetonitrile fraction at the zero time interval (phosphoric acid added to egg yolk, liver and feces of untreated hens), the P^{32} was probably incorporated into normal phosphorous-containing metabolities of the yolk, liver, or feces.

When hens received diethyl phosphorodithioic acid in the diet, there were no acetonitrile-soluble materials recovered from the yolk and very few from the liver, but the feces contained almost 1 p.p.m. of acetonitrile-soluble radioactive materials by 3 days after treatment (table 3). Most of the radioactive materials remained in the tissue residue following extraction with acetone or benzene. It was noted that when known amounts of diethyl phosphorodithioic acid were added to egg yolk, feces, and liver there was some acetonitrile-soluble radioactivity. Generally, the acetonitrile-soluble materials are assumed to be intact insecticides or oxidation products; however, these data would render such a viewpoint erroneous. The occurrence of acetonitrile-soluble materials in recovery experiments or in tissues from treated hens (table 3) could be the result from occlusion of the diethyl phosphorodithioic acid in fat particles and incomplete separation of the fat from the acetonitrile into the hexane fraction or from the incorporation of this acid into some naturally occurring metabolite that partitioned into the acetonitrile from the *n*-hexane. Residue data based on the amount of P^{32} in a particular solvent of a fractionation scheme would be misleading; characterization of the radioactive materials is essential. The P^{32} in the acetonitrile fraction of egg yolk from hens dusted with Co-Ral (Dorough et al. 1961a) or hens fed Co-Ral (Dorough et al. 1961b) were probably phosphoric acid or a metabolite containing this acid.

Table 3.—P.p.m. acetonitrile-soluble radioactive materials in liver, egg yolk, and feces from hens receiving phosphoric acid and diethyl phosphorodithioic acid in the feed.

Days After Treatment	Phosphoric Acid			Diethyl Phosphorodithioic Acid		
	Egg yolk	Liver	Feces	Egg yolk	Liver	Feces
0	<0.01	<0.01	<0.01	<0.01	<0.01	0.02
1	< .01	< .01	< .01	—	.03	.53
3	< .01	.12	< .01	< .01	.04	.92
7	.20	.01	.02	< .01	.03	.77
14	.06	< .01	< .01	—	< .01	.14

The radioactive acetonitrile-solubles of the liver from phosphoric acid-treated hens (table 3) were chromatographed on Celite using the same amount of solvent as for Co-Ral and its metabolites (Dorough et al. 1961a). Greater than 99% of the P^{32} in the acetonitrile fraction of the liver was eluted from Celite in the methanol fraction; the remainder of the P^{32} was eluted from the column in the first 75 ml. of mobile solvent. The acetonitrile-soluble P^{32} from egg yolk was about 85% in the methanol fraction and about 15% in the first 75 ml. of mobile solvent. The radioactivity in the methanol fraction was probably free phosphoric acid or phosphoric acid attached to a very labile compound, since no oxidation was necessary to produce the phosphorus color (Allen 1940). The water-soluble P^{32} materials recovered from the feces of phosphoric acid-treated hens co-chromatographed on an anion exchange column as free phosphoric acid.

The unextractable P^{32} materials in the yolk, feces, and liver residue were further separated into acid-soluble compounds, ribonucleic acid, desoxyribonucleic acid, and phospholipids (table 4). Since acetone and benzene did not remove known amounts of P^{32} phosphoric acid added to the yolk, liver, or feces residue, most of the radioactivity was in the acid-soluble fraction at the zero time interval (table 4). Phospholipids and ribonucleic acid were the predominate P^{32} phosphorus-containing materials of egg yolk. The fractionation procedure of Schneider

Table 4.—Fractionation of the unextractable P^{32}-materials from egg yolk, feces, and liver residue from hens receiving P^{32}-phosphoric acid in the diet.[a]

Tissue or Feces	Days After Treatment	P.p.m. of Total Present as[b]			
		Acid-Soluble Compounds	Phospholipids	RNA	DNA
Egg Yolk	0	0.65	0.03	0.03	0.00
	1	.00	.00	.00	.00
	3	.13	.52	1.82	.09
	7	.13	.50	.60	.01
	14	.11	.68	.78	.03
Feces	0	—	—	—	—
	1	1.75	.38	.23	.07
	3	1.79	.35	.21	.07
	7	1.51	.66	.36	.10
	14	.09	.02	.00	.00
Liver	0	.57	.12	.00	.00
	1	.44	.18	.06	.00
	3	.46	.26	.09	.01
	7	.65	.39	.23	.02
	14	.32	.17	.10	.03

[a] Hens fed P^{32}-phosphoric acid in feed at 100 p.p.m. for 7 days.
[b] Averaged from four determinations at each time interval after treatment.

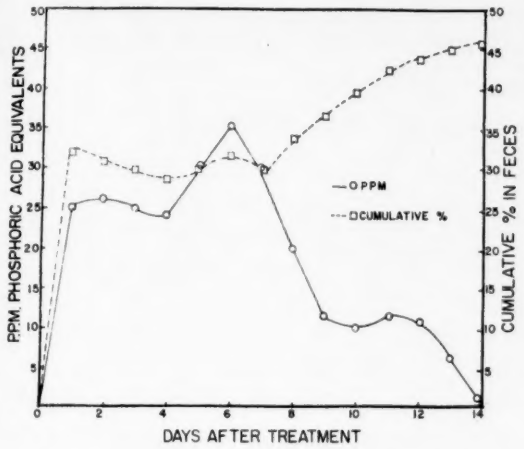

FIG. 1.—P.p.m. and cumulative percentage of phosphoric acid eliminated in the feces of hens receiving 100 p.p.m. of the radiolabeled acid in the diet.

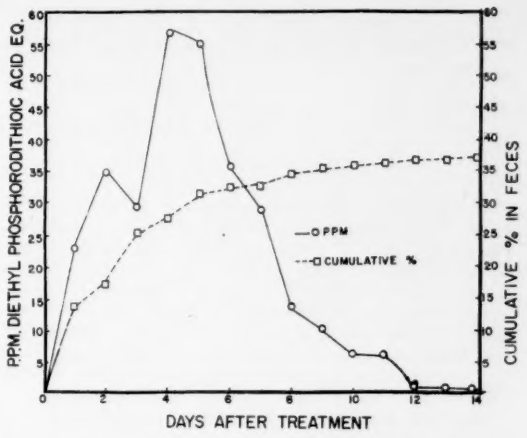

FIG. 2.—P.p.m. and cumulative percentage of *O,O*-diethyl phosphorodithioic acid eliminated in the feces of hens receiving 100 p.p.m. of the radiolabeled acid in the diet.

(1945) was efficient in removing most of the radioactive materials from the tissue residue. The P^{32} in the feces and liver residue from hens receiving phosphoric acid in the diet had the same solubility properties as observed for the unextractable P^{32} materials of the liver and feces from Co-Ral-treated hens (Dorough et al. 1961a).

Elimination in Feces.—About 46% of the phosphoric acid and 37% of the diethyl phosphorodithioic acid consumed in the feed were eliminated in the feces during the first 14 days after initiating the experiment (figs. 1 and 2). These percentages were intermediate between Co-Ral (76%) and Ruelene (30%) eliminated in the feces of laying hens (Dorough et al. 1961b, Buttram & Arthur 1961).

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ERRATUM

"Studies on fine-particle, coarse-particle and concentrated aerosols." Milton S. Schechter, William N. Sullivan, and Alfred H. Yeomans. Jour. Econ. Ent. 53(5): 908–14. Oct. 1960.

The mass median particle sizes (m.m.d.) of the aerosols given under "Materials Tested" (page 909) are correct except for formulation XI which produces aerosols of 10 microns m.m.d. instead of 20 microns m.m.d. Discrepancies in the particle sizes mentioned in the rest of the article for some of the formulations should be corrected to conform to those given under "Materials Tested."

Field Tests with Insecticides to Control Spotted Alfalfa Aphid in Southwestern Kansas, 1955–1960[1]

LESTER J. DEPEW,[2] *Kansas Branch Agricultural Experiment Station, Garden City*

ABSTRACT

All foliar sprays reduced populations of the spotted alfalfa aphid, *Therioaphis maculata* (Buckton), but only parathion, Phosdrin®, Trithion® consistently gave high initial control. Malathion and UCC 8305 were effective in one test but gave slightly below 95% control in another. Guthion®, Kelthane®, and demeton did not quite reach the effective control level. Lindane, DDT-toxaphene, Diazinon®, Thiodan®, Dibrom® and Sevin® did not provide satisfactory control. Trithion maintained very effective control at least 7 days, indicating long residual toxicity.

Alfalfa seed treated with 1 or 2 pounds actual phorate per 100 pounds of seed was relatively ineffective in controlling aphids 42 days after seeding.

Both phorate and Di-Syston® applied as granules at 1 pound actual per acre suppressed aphid populations approximately 2 weeks after treatment, but phorate definitely was inferior to Di-Syston after 4 weeks.

Chemical Definitions of Proprietary Materials Tested

Diazinon—*O,O*-dimethyl-*O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate
Dibrom—1,2-dibromo-2,2-dichloroethyl dimethyl phosphate
Di-Syston—*O,O*-diethyl *S*-2-(ethylthio)ethyl phosphorodithioate
Guthion—*O,O*-dimethyl *S*-(4-oxo-1,2,3-benzotriazine-3-(4H)-ylmethyl) phosphorodithioate
Kelthane—1,1-bis(*p*-chlorophenyl)-2,2,2-trichloroethanol
Phosdrin—1-methoxycarbonyl-1-propen-2yl dimethyl phosphate
Sevin—1-naphthyl *N*-methyl-carbamate
Thiodan—6,7,8,9,10,10-hexachloro-1,5,5*a*,6,9,9*a*-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide
Trithion—*S*-(*p*-chlorophenylthio) methyl *O,O*-diethyl phosphorodithioate
UCC 8305—*P*-chloro-2,4-dioxa-5-methyl-*P*-thionoe-3-phosphabicyclo(4.4.0) decane

The spotted alfalfa aphid, *Therioaphis maculata* (Buckton), was first collected in Kansas by the writer from seedling alfalfa in Stanton County in August, 1954. By fall 1955, it was in nearly all Kansas counties.

According to Burkhardt (1959), spotted alfalfa aphids cost Kansas an estimated 2.4 and 8.5 million dollars during 1955 and 1956, respectively. Subsequent years have been less favorable for high populations of this insect in Kansas.

Experimental work with insecticides for control of spotted alfalfa aphids have been published by Reynolds & Anderson (1955), Stern & Reynolds (1957), Randolph (1957), Dobson & Watts (1957), Stern & Reynolds (1958), Dobson (1958), Bishop & Burkhardt (1959), Burkhardt (1959), and Roth (1959). The effects of various pressures, high and low gallonages, particle size of droplets at high and low temperatures on aphid control in Kansas were reported by Burkhardt (1957). The present paper is a report of work conducted in southwestern Kansas over a 5-year period.

MATERIALS AND METHODS.—The chemical definitions of proprietary materials referred to in this paper are given at the end of the paper preceding "References Cited." All insecticides tested as sprays were emulsions except Sevin, which was an 85% wettable powder. Insecticides and rates used are shown in tables 1–4. Because the reproductive habits of the spotted alfalfa aphid make possible tremendous increases in population in a relatively short time, 95% kill or better was used as the criterion for considering a material adequately effective.

TEST NO. 1.—Sprays were applied on alfalfa plots near Larned, Kansas. The alfalfa was about 8 inches tall. The insecticides were applied with a compressed-air hand sprayer equipped with an 18-inch boom having three cone-type nozzles. The sprayer was operated at a pressure of 20 p.s.i. and delivered approximately 14 gallons per acre. Each treatment was replicated four times in a randomized block design, each replicate was 50 by 50 feet in area.

Aphid populations were estimated 4 and 10 days after application by Henderson's sampling fork method (1956). The efficiency of the various insecticidal treatments was determined by reduction of aphids compared with the untreated check.

TEST NO. 2.—On September 14, 1956, sprays were applied to alfalfa plots near Holcomb, Kansas. The alfalfa was about 6 inches tall. Sprays were applied with a compressed-air hand sprayer, which operated at a pressure of 30 p.s.i. and delivered approximately 18 gallons per acre through three cone-type nozzles. Each treatment was replicated three times in a randomized block design; each replicate was 25 by 25 feet in area. The spray materials were evaluated 1, 3 and 7 days after application as described above.

TEST NO. 3.—Another test was conducted in the same area (near Holcomb). The alfalfa was approximately 12 inches tall. In this experiment a tractor-mounted sprayer was used, equipped with an 8-foot boom containing five cone-type nozzles arranged for broadcast spraying. The sprayer was operated at a pressure of 40 p.s.i. and delivered 14 gallons per acre. Each treatment was replicated four times in a randomized block design. Each replicate was 54 by 100 feet in area. Treatments were evaluated 3 and 10 days after application.

TEST NO. 4.—This experiment was designed to evaluate the effectiveness of a systemic insecticide as a seed treatment on alfalfa seedlings.

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[2] Assistant Entomologist, Kansas Agricultural Experiment Station.

Table 1.—Results of foliar spray tests to control spotted alfalfa aphids in southwestern Kansas.

Material	Actual Toxicant per Acre (lb.)	First Sample		Second Sample		Third Sample	
		No. of Aphids[a]	Reduction (%)	No. of Aphids[a]	Reduction (%)	No. of Aphids[a]	Reduction (%)
		Interval between Treatment and Sampling					
Test No. 1—1955		*4 Days*		*10 Days*			
Parathion	0.25	8.3	96	3.3	85		
Demeton	0.25	22.3	90	4.0	80		
Malathion	0.75	8.0	96	4.5	80		
Lindane	0.25	50.0	76	7.8	60		
Check	—	221.5	—	20.0	—		
L.S.D. at 5% level		18.1		6.0			
at 1% level		25.4		8.4			
Test No. 2—1956		*1 Day*		*3 Days*		*7 Days*	
Parathion	0.25	19.0	95	15.3	96	49.3	83
Phosdrin	0.25	6.3	98	2.3	99	17.0	94
DDT-toxaphene	1.0–1.0	212.7	39	63.7	95	70.7	75
UCC 8305	0.25	10.7	97	5.0	99	28.0	90
Check	—	351.0	—	416.7	—	285.7	—
L.S.D. at 5% level		31.9		25.0		30.3	
at 1% level		46.4		36.4		44.1	

[a] Average per fork sample.

Vernal alfalfa (susceptible) was planted in randomized plots at the Garden City Experiment Station on September 13, 1958. Plots were one drill width by 100 feet in length. The alfalfa seed had been commercially treated by the American Cyanamid Company at 1 or 2 pounds of actual phorate per 100 pounds of seed. The seed also was treated with a fungicide, Captan 50W, at 3 ounces actual toxicant per 100 pounds of seed.

Aphid populations were estimated by Dobson's method (1958) and each plot was sampled twice.

TEST NO. 5.—This experiment was designed to determine the effectiveness of granulated insecticides in reducing aphid populations on alfalfa seedlings.

Buffalo alfalfa was planted August 17, 1960, at the Garden City Experiment Station. Plots were each 7½ by 165 feet in a solid block and surrounded on all sides by newly seeded, untreated alfalfa. Treatments were replicated four times. Granulated phorate and Di-Syston were applied at approximately 1 pound actual per acre directly to the seed furrow at time of planting. No precipitation fell so plots were flood irrigated 9 days after seeding.

Aphid populations were estimated by periodic counts of apterous aphids on 4 feet of row selected at random in each plot.

TEST NO. 6.—On August 26, 1960, eight sprays were applied to 6-inch established alfalfa using a tractor-mounted sprayer with an 8-foot boom set for broadcast spraying. The sprayer was operated at a pressure of 40 p.s.i. and delivered 13.4 gallons per acre. Each treatment was replicated four times in a randomized block design; each replicate was 36 by 100 feet in area.

Treatments were evaluated 3, 7, and 20 days after application by counting the number of apterous aphids on 25 stems in each replicate; this gave a total of 100 stems per treatment.

RESULTS AND DISCUSSION.—In test 1 (table 1), parathion and malathion gave 96% control of the spotted alfalfa aphid 4 days after application. Demeton was quite effective in reducing the degree of infestation (90%), but lindane was definitely inferior to the above insecticides. As a result of cool, damp weather and/or cutting of the alfalfa around the plot area, aphid populations decreased markedly 10 days after application and no material approached the level of being adequately effective.

In test 2 (table 1), Phosdrin, UCC 8305, and parathion gave adequate protection 1 day after application. Three days after application, all three materials still held the aphid in check. It will be noted that aphid control resulting from the DDT-toxaphene spray increased from 39% to 95% between the 1-day and 3-day counts. As a result of predators, particularly lady beetles and lacewings, reduced populations were apparent at the 7-day interval. At this time none of the materials reached the 95% level.

In test 3 (table 2), Phosdrin, parathion, and Trithion provided 95% to 97% control 3 days after application. Kelthane provided 91% but UCC 8305, contrary to test 2, gave only 90% control. Trithion continued to provide 95% control 10 days after application but control had decreased in the Phosdrin-treated plots. None of the other treatments gave satisfactory control 10 days after application.

In test 4 (table 3), aphid populations increased rather slowly so that appreciable numbers did not appear until mid-October. At the 42-day interval, aphid control with

Table 2.—Results of foliar spray tests to control spotted alfalfa aphids in southwestern Kansas.

Material	Actual Toxicant per Acre (lb.)	Interval Between Treatment and Sampling: 3 days — No. of Aphids[a]	3 days — Reduction (%)	10 days — No. of Aphids[a]	10 days — Reduction (%)
Test No. 3—1957					
Parathion	0.25	8.0	95	21.0	84
Phosdrin	0.25	5.3	97	11.8	91
DDT-toxaphene	1.0–1.0	24.8	85	39.0	71
Diazinon	0.5	17.0	89	28.3	79
Trithion	0.25	6.0	96	7.0	95
Kelthane	0.5	15.3	91	29.8	77
UCC 8305	0.5	16.0	90	18.0	85
Check	—	166.8	—	133.3	—
L.S.D. at 5% level		18.8		9.3	
at 1% level		25.7		12.7	

[a] Average per fork sample (composite of 4 replications).

phorate at 1 or 2 pounds per acre was poor. Stand counts taken 16 days after seeding showed that phorate reduced seedling stand 26% and 28% at 1 and 2 pounds, respectively.

In test 5 (table 3), Di-Syston and phorate at 1 pound actual per acre were equally effective in suppressing aphid populations for 15 days. Then, phorate began to lose its effectiveness while loss of effectiveness by Di-Syston was comparatively slower. Forty-two days after application neither material was effective. A 1.62-inch rain had markedly reduced aphid populations before the 42-day count. Data taken September 8 showed no reduction in seedling alfalfa stands from Di-Syston and phorate granules when applied in furrows at seeding. Plant growth definitely was retarded by aphid feeding in untreated check plots.

In test 6 (table 4), Phosdrin, Trithion, and methyl parathion provided 96% to 97% control 3 days after application. The other materials were inferior to the above insecticides, although malathion and Guthion approached the level of being adequately effective. Trithion maintained 95% control through the seventh day. All other materials dropped below the 95% level 7 days after application. None of the insecticides provided adequate control 20 days after application. As a result of predators and/or spraying of surrounding alfalfa, aphid populations dropped sharply.

The chemical names of proprietary materials mentioned in this article are as follows:

Diazinon—*O,O*-dimethyl-*O*-(2-isoprophy-4-methyl-6-pyrimidinyl) phosphorothioate
Dibrom—1,2-dibromo-2,2-dichloroethyl dimethyl phosphate
Di-Syston—*O,O*-diethyl *S*-2-(ethylthio)ethyl phosphorodithioate
Guthion—*O,O*-dimethyl *S*-(4-oxo-1,2,3-benzotriazine-3-(4H)-ylmethyl) phosphorodithioate
Kelthane—1,1-bis(*p*-chlorophenyl)2,2,2-trichloroethanol
Phosdrin—1-methoxycarbonyl-1-propen-2yl dimethyl phosphate
Sevin—1-naphthyl N-methylcarbamate
Thiodan—6,7,8,9,10,10-hexachloro-1,5,5*a*,6,9,9*a*-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide
Trithion—*S*-(*p*-chlorophenylthio) methyl *O,O*-diethyl phosphorodithioate
UCC 8305—*P*-chloro-2,4-dioxa-5-methyl-*P*-thiono-3-phosphabicyclo(4.4.0) decane

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Table 3.—Results of seed treatment and granular formulations applied to the soil to control spotted alfalfa aphids on seedling alfalfa, Garden City, Kansas.

Material	Toxicant (lb.)	First Sample — No. of Aphids[a]	First Sample — Reduction (%)	Second Sample — No. of Aphids[a]	Second Sample — Reduction (%)	Third Sample — No. of Aphids[a]	Third Sample — Reduction (%)	Fourth Sample — No. of Aphids[a]	Fourth Sample — Reduction (%)
		Interval between Treatment and Sampling							
Test No. 4—1958		*22 Days*		*42 Days*					
Phorate	2.0[b]	—[c]	—	24.1	77				
Phorate	1.0[b]	—[c]	—	79.2	24				
Check	—	—[c]	—	105.0	—				
L.S.D. at 5% level				9.4					
Test No. 5—1960		*15 Days*		*22 Days*		*30 Days*		*42 Days*	
Di-Syston	1.0[d]	1.5[e]	99	4.8[e]	98	14.5[e]	91	1.3[e]	76
Phorate	1.0[d]	2.5[e]	98	35.0[e]	86	57.5[e]	65	3.8[e]	28
Check	—	152.8[e]	—	247.0[e]	—	165.5[e]	—	5.3[e]	—
L.S.D. at 5% level		42.2		54.5		14.3		2.8	
at 1% level		63.9		82.6		21.6		4.3	

[a] Average per linear foot of row (composite of 4 replications). Both apterous and alate forms counted.
[b] Actual per 100 pounds of seed.
[c] Only trace infestation observed in plots.
[d] Actual per acre applied as granules in seed furrow.
[e] Only apterous forms counted.

Table 4.—Results of foliar spray treatments applied by ground equipment to control spotted alfalfa aphids in established alfalfa, Garden City, Kansas.

Material	Actual Toxicant per Acre (lb.)	Interval between Treatment and Sampling: 3 Days — No. of Aphids[a]	3 Days — Reduction (%)	7 Days — No. of Aphids[a]	7 Days — Reduction (%)	20 Days — No. of Aphids[a]	20 Days — Reduction (%)
Test No. 6—1960							
Phosdrin	0.25	8.5	97	65.3	72	51.3	10
Trithion	0.5	10.8	96	12.5	95	19.0	67
Methyl parathion	0.25	10.0	96	20.3	91	17.5	69
Malathion	1.0	16.8	93	23.3	90	20.8	64
Guthion	0.5	21.3	91	35.8	85	24.5	57
Thiodan	0.75	35.0	86	45.8	80	27.3	52
Dibrom	1.0	38.0	84	78.8	66	31.3	45
Sevin	1.0	85.5	65	120.8	48	28.0	51
Check	—	244.3	—	231.5	—	57.3	—
L.S.D. at 5% level		29.4		23.6		17.3	
at 1% level		39.8		32.0		23.4	

[a] Average per 25 stems (composite of 4 replications).

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Experiments on Control of the Face Fly[1]

J. H. FALES, J. C. KELLER, and O. F. BODENSTEIN,[2] *Entomology Research Division, Agric. Res. Serv., U.S.D.A.*

ABSTRACT

Field tests were conducted in central Maryland against the face fly (*Musca autumnalis* DeGeer), which occurred for the first time in this area as a serious pest of livestock in 1960. Baits containing sugar syrup or malt plus Dipterex® (dimethyl(2,2,2-trichloro-1-hydroxyethyl)phosphonate), methoxychlor, DDVP, and malathion were compared in tests in which the face, head, or topline of the animal was treated. Dipterex and DDVP gave erratic reduction but were superior to methoxychlor and malathion. Pyrethrins and piperonyl butoxide in mineral oil with and without repellent R-326® (di-*n*-propyl isocinchomeronate) and barthrin and piperonyl butoxide were sprayed on the head and the head and front quarters of the dairy and beef cattle. Only barthrin-piperonyl butoxide was moderately effective into the second day. Other tests compared repellent effects of various materials applied to the heads of the animals with paintbrushes. Several sprays containing pyrethrins plus synergists or repellents were also tested. Repellent R-1207® (3-chloropropyl *n*-octyl sulfoxide) was most effective 1 day after treatment. Emulsions and dusts tested were ineffective.

In 1960 the face fly (*Musca autumnalis* DeGeer) became a serious pest of cattle and horses in central Maryland for the first time (Plant Pest Control Division, U.S.D.A., 1960) (see figs. 1 and 2). This situation prompted workers at Beltsville to begin investigating control of this pest, to observe its field biology, and to rear the insect in the laboratory (Fales et al. 1961). The tests reported in this paper were conducted in the field in Prince Georges, Montgomery, and Howard Counties to evaluate different insecticides, synergists, repellents, combinations of these, and poison baits for the control of the fly on livestock.

A few of the results represent unreplicated tests but are included because of the widespread interest in control

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FIG. 1.—Holstein cow showing face flies on jaw, brisket and foreleg.

FIG. 2.—Holstein cow showing face flies on head.

of this fly. Treece (1960) and Matthysse (1961) have published the results of their work on face fly control.

RESULTS.—*Baits.*—The poison baits were formulated as solutions or suspensions of insecticide in 80% sugar syrup or malt extract and applied daily to the faces of livestock with a paintbrush (fig. 3) or a pump-type oilcan or to the heads and topline with the oilcan. Effectiveness was based on fly counts before and for different intervals after treatment and compared with a check herd and adjusted by Abbott's formula. The results of these tests are summarized in table 1.

In general, baits containing malathion or methoxychlor applied daily to the face caused unsatisfactory reductions on dairy cows, even though the baits killed large numbers of flies. Four or five milliliters of Dipterex® (dimethyl (2,2,2-trichloro-1-hydroxyethyl) phosphonate) bait applied to the faces of beef or dairy animals produced erratic but generally unsatisfactory results. Ten milliliters applied to the head and topline of dairy cattle with a pump oilcan produced nearly 90% reduction at the first application. However, on beef cattle, high reductions did not occur until the seventh day. When 15 ml. was used on beef cattle, 96% reduction was obtained after the seventh treatment. DDVP bait at 2 ml. per treatment failed to reduce flies, but 5 ml. resulted in fairly good but erratic control. Bruce et al. (1960) reported that 3 ml. per head of a 0.2% DDVP bait eliminated most of the flies. In the Maryland tests Dipterex at 2 or 3 ml. and DDVP at 2 ml. per treatment gave excellent control on a Welch pony isolated from breeding areas by about one-fourth mile.

Insecticide Sprays.—Mineral oil sprays were applied to the heads of the animals with a 1-gallon hand-pressurized sprayer (fig. 4). After pretreatment counts, an entire herd was treated with a single spray. Posttreatment counts were always compared with an untreated herd. Results are given in table 2.

Table 1.—Effectiveness of daily treatments with baits applied to faces of livestock for control of face flies.

INSECTICIDE, CONCENTRATION (%), AND ATTRACTANT	APPROXIMATE AMOUNT OF BAIT PER ANIMAL (ML.)	NO. OF ANIMALS TREATED	METHOD OF APPLICATION	PER CENT REDUCTION OF FLIES AFTER DAYS SHOWN						
				1	3	5	7	10	14	21
			Dairy Cows							
Dipterex, 0.8, sugar syrup	5	36	Oilcan	32	38	51	81	76	83	—
	5[a]	22	Paintbrush	0	4	11	51	38	34	21
	10[b]	36	Oilcan	89	97	91	94	91	—	—
malt extract	5[a]	22	Paintbrush	17	0	28	31	—	—	—
Methoxychlor, 1.6, sugar syrup	6[a]	22	Paintbrush	29	40	9	7	31	21	—
DDVP, 0.1., malt extract	5[a]	22	Paintbrush	71	93	97	89	76	81	—
sugar syrup	2	32	Paintbrush	—	12	0[c]	0	0[d]	43	81
Malathion, 1.6, sugar syrup	9	47	Paintbrush	—	84	80	61	0	33	32
			Beef Cattle							
Dipterex, 0.8, sugar syrup	4	18	Oilcan	23	58	66	76	61	58	—
	10[b]	18	Oilcan	19	27	20	88	91	91	—
	15[b]	31	Oilcan	7	66	61	96	—	—	—
			Pony							
Dipterex, 0.8, sugar syrup	2 or 3	1	Paintbrush	56	86	90	90	89	96	0
DDVP, 0.1, sugar syrup	2	1	Paintbrush	88	96	98	—	—	—	—

[a] Cows licked bait from faces and were in very lush, tall pasture.
[b] Applied to face and topline.
[c] Check herd dusted by owner prior to count.
[d] Check herd grazing in Sudan grass at time of count.

FIG. 3.—Bait being applied to dairy cow by paint brush.

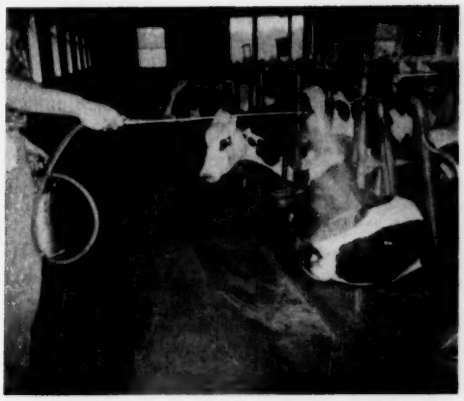

FIG. 4.—Dairy cow being treated with mineral oil spray.

The spray containing 0.1% of pyrethrins and 1% of piperonyl butoxide was evaluated first because it had recently been registered for use against face flies on cattle and was available to cattlemen. This spray was applied to mixed-breed heifers at the Agricultural Research Center, Prince Georges County. A high reduction of flies was obtained for 2 full days. Because results were promising, concentrations of the insecticides in the spray were reduced by half, but this spray was ineffective 1 day after treatment.

The first spray was then retested on a herd of lactating Holstein cows 25 miles west of Beltsville, at Rockville in Montgomery County, where the face fly population was much heavier. There this spray, which had shown promise in the lighter infestations at Beltsville, resulted in only a 17% reduction in flies less than 1 day after an evening spraying.

The other spray tests were made on a larger herd of Hereford beef cattle at Germantown in Montgomery County. The same spray used at Rockville, but with 1% of MGK Repellent 326® (di-*n*-propyl isocinchomeronate) added, was applied to the heads of the cattle while the animals were confined at the end of a chute. Two hours after the last animal was treated and released from the chute, 88% reduction in flies was recorded. However, after 24 hours no reduction was observed. Twenty-two days later the same herd was treated with a spray containing 2% of barthrin plus 1% of piperonyl butoxide on their heads, necks, and front quarters including front legs. A moderate reduction of flies occurred through the second day. This formulation, tested 14 days later with the same results, appeared to give greater relief than the one with pyrethrins, piperonyl butoxide, and MGK Repellent 326, possibly because more areas of the animals were treated.

A spray containing barthrin alone at a concentration of 2% was tested on a small herd of Holstein heifers at Beltsville. Data obtained are not included in the table because counts on the untreated check herd were too variable. However, the treated herd had only 0.3 fly per head at 7 hours after treatment and 0.5 per head after 1 day.

A liquefied-gas-propelled residual spray was also evalu-

Table 2.—Effectiveness of insecticide sprays applied to heads or heads and front quarters of livetsock for control of the face fly.[a]

Material in Mineral Oil	Concentration (%)	Dosage per Head (oz.)	Per Cent Reduction of Flies in Treated Herd							Number of Flies per Head in Untreated Herd						
			Number of Animals	Days After Treatment						Number of Animals	Days After Treatment					
				½	1	2	3	7	14		½	1	2	3	7	14
Pyrethrins	0.1	2.1	46	97	96	87	29	74	73	26	9	1	8	2	3	2
Piperonyl butoxide	1.0															
Pyrethrins	0.1	2.7	34	—	17	51	0	0	0	6	—	30	26	13	6	—
Piperonyl butoxide	1.0															
Pyrethrins	0.05	2.0	34	54	0	6	18	77	42	20	1	1	2	1	4	3
Piperonyl butoxide	0.50															
Pyrethrins	0.1	2.8	80*	—	0	0	0	0	0	28	—	5	7	7	16	12
Piperonyl butoxide	1.0															
MGK Repellent 326	1.0															
Barthrin	2.0	3.6	82*	—	80	56	0	22	—	58	—	9	6	5	11	1
Piperonyl butoxide	1.0															

[a] Single herd treatments except barthrin and piperonyl butoxide average of two. Dairy cattle, except beef cattle where indicated by asterisk.

ated. Results from the formulation are not shown in the table because it was not effective. This spray contained 0.05% of pyrethrins, 0.5% of piperonyl butoxide, 0.2% of DDVP, 6.2% of Dri-Die (silica gel), 39.55% of solvents, 28.5% of Propellent-11, and 25% of Propellent-12.

The Holstein dairy herd at Rockville was used in another study to determine how often it had to be sprayed to obtain continued relief. The spray containing 0.1% of pyrethrins and 1% of piperonyl butoxide was applied to the heads of the animals of the entire herd on 11 days during the period from August 8 to September 1. The dosages used on August 8 and 11 were 6.8 and 5.7 ounces, respectively, and the heads and front quarters of the animals were treated. The dosage applied on August 16 to the same areas of the animals was not determined. The average dosage of the remaining eight treatments was 2.8 ounces applied to the head only. The results given below indicate that daily treatments are necessary. However, if treatments were made in the morning every other day, a certain amount of relief could still be expected on the second day, but much less than on the first.

Date[a]	Reduction of Flies (%)	Check Population (per head)	Date[a]	Reduction of Flies (%)	Check Population (per head)
August 8*	93	21	August 20*	—	—
9	39	12	22*	92	26
10	0	5[b]	23	52	12
11*	47	8	24*	68	7
12	19	10	25	24	2
15	0	17	26*	92	17
16*	79	11	28*	—	—
17	0	9	29	0	18
18*	24[b]	5	30*	—	—
19	58[c]	18	31	63	13
			Sept. 1	—	—
			2*	67	40

[a] * indicates treatment applied.
[b] Herd in open shed.
[c] Abnormal count, animals moving.

Brush-on Treatments.—Surveys in September in Montgomery County at Rockville and Redland showed that fairly heavy populations of face flies were still present; testing was therefore continued. Crag Fly Repellent® (butoxy polypropylene glycol), MGK Repellent 326, MGK Repellent 1207® (3-chloropropyl *n*-octyl sulfoxide), piperonyl butoxide, and Tabutrex® (di-*n*-butyl succinate) were tested separately at a concentration of 5% in mineral oil. A formulation containing 0.1% of pyrethrins and 1% of piperonyl butoxide was again tested, but as an emulsion containing 1.26% of polyoxyethylene sorbitol ether ester and 6.1% of petroleum distillate. A formulation containing 0.06% of pyrethrins, 0.12% of piperonyl butoxide, 0.2% of MGK-264® ((*N*-2-ethylhexyl)bicyclo-[2.2.1]hept-5-ene-2,3-dicarboximide), and 0.4% of MGK Repellent 326 was also tested as well as a heavy wipe-on material containing 0.1% of pyrethrins, 1% of piperonyl butoxide, 5% of Crag Fly Repellent, 5% of Atlox 8916T (polyoxyethylene sorbitan esters of mixed fatty and resin acids), 2% of sodium carboxymethyl cellulose, 0.4% of petroleum distillate, and 86.5% of water. Each of these formulations was generously applied with a 2-inch paintbrush to the heads of three animals in a given herd. Untreated animals in the same herd served as checks. The tests were repeated on another herd. Results of both tests are given in table 3.

Table 3.—Effectiveness against face flies of 5 repellents and 3 insecticides applied by paintbrush to heads of dairy cattle at dosages of 2 to 3 ounces per animal.[a]

Material in Mineral Oil	Concentration (%)	Per Cent Reduction of Face Flies per Head: Days After Treatment						
		0.25	1	2	3	4	5	6
Crag Fly Repellent	5	81	50	—	29	22	50	100
		76	11	0	—	—	—	—
MGK Repellent 326	5	96	72	—	45	41	0	78
		91	62	26	—	0	—	—
MGK Repellent 1207	5	61	83	—	16	11	0	0
		60	86	0	—	0	—	—
Piperonyl butoxide	5	73	56	—	0	52	50	78
		100	41	45	—	0	—	—
Tabutrex	5	69	64	—	37	81	70	11
		—	—	—	—	—	—	—
Pyrethrins[b] Piperonyl butoxide	0.1 1.0	61	0	—	0	11	40	11
		60	24	16	—	21	—	—
Pyrethrins[c] Piperonyl butoxide Crag	0.1 1.0 5	67	42	—	50	0	0	11
		76	71	3	—	21	—	—
Pyrethrins Piperonyl butoxide MGK-264 MGK Repellent 326	0.06 0.12 0.2 0.4	69	61	—	3	22	40	0
		67	74	0	—	0	—	—
		Average number of flies at indicated days						
Untreated check animals		13.9	7.4	—	7.9	5.6	2.2	1.8
		4.2	14.1	2.7	—	1.7	—	—

[a] Upper figures Redland and lower figures Rockville.
[b] Water emulsion.
[c] Heavy wipeon.

MGK Repellent 1207 was the most effective material 1 day after treatment. MGK Repellent 326 was also moderately effective. Piperonyl butoxide caused a fair reduction of flies. The spray containing Crag Fly Repellent alone was effective at 6 hours but not after 1 day. Tabutrex alone, which was tested on only one herd, also caused a fair reduction that was the same at 1 day as at 6 hours. The MGK spray and the heavy wipeon treatment were interesting in that they were almost as effective at 1 day as at 6 hours, although not so effective as some of the repellents alone. The emulsion was the least effective material tested in the brushon tests. None of the materials tested were effective beyond 1 day. Some of the data, however, show sporadic reductions in flies at later counts, but these were unreliable, since populations in the checks declined.

Wipeon Treatments.—Other tests were made with a formulation containing 0.1% of pyrethrins, 1.0% of piperonyl butoxide, and 5% of Crag Fly Repellent applied as individual wipeon treatments on several occasions to dairy cattle, beef cattle, and horses. The results of these tests, not given here, were erratic because of the lack of suitable untreated check herds. The owners felt, however, that the animals were temporarily relieved from attacks by the face fly.

Dust Treatments.—An attempt was made to evaluate a 4% malathion dust. One dairyman agreed to apply the material every morning. The flies were not reduced by this method because the dairyman would not apply the

heavy dosage considered necessary. Thus the daily dusting was discontinued.

CONCLUSIONS.—In general, baits did not reduce the flies. The following factors might have contributed to their failure:

(1) Treatments were not begun early enough in the season;

(2) Animals licked the baits from each other;

(3) Animals were pastured in tall, lush, dew-laden fields, so that the insecticide was rubbed off before the flies could come in contact with the bait;

(4) All or a substantial number of the animals on the premises were not treated.

In the spray and wipeon tests no outstanding material or formulation was found, but the following conclusions can be drawn:

(1) Temporary relief can be obtained with many of the materials tested, but little, if any, for more than 24 hours after treatment;

(2) Sprays containing both barthrin and piperonyl butoxide can be expected to give good-to-fair control longer than any of the other materials tested;

(3) Increased amounts of certain repellents alone may be as useful as sprays which contain two or more ingredients;

(4) Sprays should be applied to dairy animals after the morning and not the evening milking;

(5) Sprays are easier to apply than the wipeon or the brushon treatments;

(6) Mineral-oil type formulas would be satisfactory on a daily basis for dairy cows but would not be practical for beef cattle.

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Effect of Insecticides on the Sugar-Beet Root Aphid, *Pemphigus betae*[1]

A. M. HARPER[2]

ABSTRACT

Aldrin, lindane, and parathion reduced numbers of the sugar-beet root aphid, *Pemphigus betae* Doane, on sugar beets in greenhouse tests. In field tests there was an increase in yield of beets in both 1958 and 1959 at one of two locations when aldrin and parathion were applied to the soil. The increase appeared to result from aphid control early in the season. Both insecticides reduced the populations of the predator, *Thaumatomyia glabra* Meigen. By fall the large numbers of *T. glabra* present in the untreated plots had reduced the numbers of aphids to levels equal to or lower than those of insecticide-treated plots.

Lange et al. (1957) reported that a soil application of parathion controlled the lettuce root aphid, *Pemphigus bursarius* L., in California. Dunn (1960) found that the same species of aphid could be controlled in England by using a seed dressing of endrin followed by an endrin or Diazinon spray applied along the row just after the lettuce had been singled. Endrin or Diazinon worked into the top 3 inches of soil before planting, or applied as sprays during the growing season also controlled the aphid. This paper reports on greenhouse and field experiments with several insecticides for control of the sugar-beet root aphid, *Pemphigus betae* Doane, in southern Alberta.

METHODS AND MATERIALS.—Two experiments were conducted in the greenhouse in 1955 to determine the influence of various insecticides on populations of the sugar-beet root aphid reared on young growing sugar beets in individual pots. Aldrin, chlordane, DDT, dieldrin, endrin, heptachlor, lindane, malathion, and parathion were included in the first experiment. On March 17 all the insecticides were applied to the soil (pH 7.8) at a rate of 5 pounds of toxicant per acre except lindane, which was applied at 1 pound per acre. Each pot was seeded after the treatments were applied. Six weeks later each beet was infested with 20 mature aphids of uniform size. Resulting populations of aphids were determined 1 month after the beets were infested. In the second experiment toxaphene was used instead of DDT and all the insecticides were applied at a rate of 5 pounds of toxicant per acre on June 29. The beets were infested as previously described and the numbers of aphids in each pot were determined 2 months after the beets were infested. The pots in each test were distributed on greenhouse benches in 10 randomized blocks. Logarithmic transformation was applied to the data before significance was tested by analysis of variance.

In 1958 and 1959, aldrin and parathion, two of the most promising insecticides in the greenhouse experiments, were tested in sugar-beet fields at Monarch and Coaldale, Alberta, to determine their influence on naturally occurring populations of *P. betae*. The plots at Monarch were located in a field of loam soil in 1958 and clay loam in 1959, and the plots at Coaldale were located on silty clay loam soils in both years. The pH of the soils was 8.0. In 1958 the plots, each 8 rows wide and 300 ft. long, were set out in 4 randomized blocks whereas in 1959 they were each 8 rows wide and 100 feet long and arranged in 8 randomized blocks. The insecticides were sprayed on the soil at a rate of 5 pounds of toxicant per acre with a boom sprayer that delivered 13 gallons of liquid per acre. In 1958 the insecticides were applied on

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[2] Entomologist, Research Station, Lethbridge.

April 29 at Monarch and on May 4 at Coaldale and in 1959 on May 16 and May 23, respectively. In both years the insecticides were harrowed into the upper 3 inches of soil immediately after spraying and the beets were planted the day after the treatments were applied.

Stands were determined before thinning in 1958 by counting all the beets in the four central rows of each plot and in 1959 by counting the "beet-containing" inches in 100 in. in each of the four central rows. In 1958, on July 30 at Monarch and August 4 at Coaldale 10 beets in each of the two central rows of each plot were rated from 0 to 3 for aphid infestation on the basis of 0 for no infestation and 3 for a severe infestation. To determine stand, yield, and aphid infestation in the fall all the beets in 50 feet of each of the four central rows of each plot were used in 1958 and all the beets in 25 feet of each of the four central rows in 1959. The percentage sugar was determined from two 25-beet samples harvested from each plot.

At Monarch, in 1959, counts were made in the fall of the larvae and pupae of the predator, *Thaumatomyia glabra* Meigen, found in the soil around two beets selected at random in each plot; each soil sample was 1 square foot in size and centered on a beet.

RESULTS AND DISCUSSION.—In the first experiment in the greenhouse, soil treated with parathion, aldrin, lindane, endrin, heptachlor, and dieldrin contained significantly fewer aphids than the untreated soil 1 month after infestation (table 1). In the second greenhouse experiment in which the population was allowed to build up for 2 months the numbers of aphids were significantly less in soil treated with parathion, lindane, and aldrin than in the untreated soil (table 1). Although dieldrin, endrin, and heptachlor controlled the aphids 1 month after infestation, they did not prevent a subsequent increase in their numbers whereas aldrin, lindane, and parathion continued to be effective for at least 2 months.

In studies in the field in 1958 and 1959, aldrin and parathion did not appear to be phytotoxic as the emergence and subsequent stand of sugar beets was not significantly different in treated and untreated plots (table 2).

Table 1.—Numbers of sugar-beet root aphids on sugar beets grown in the greenhouse in soil treated with insecticides at the rate of 5 pounds of toxicant per acre.

TREATMENT	MEAN NUMBER OF APHIDS PER BEET	
	Experiment I	Experiment II
	1 Month After Beets Infested[a]	2 Months After Beets Infested[a]
Parathion	0*	0*
Aldrin	3.6*	2.1*
Lindane[b]	3.9*	2.1*
Endrin	11.8*	386.9
Heptachlor	26.2*	902.1
Dieldrin	83.7*	389.3
Chlordane	152.8	399.6
Malathion	201.5	396.0
Toxaphene	—	471.6
DDT	325.7	—
Untreated	229.3	521.7

[a] Initial infestation 20 aphids/beet.
[b] Applied at 1 lb. per acre in Experiment I.
* Significantly lower than the untreated at the 1% level.

Table 2.—Mean stand and yield of sugar beets and mean infestation rating in plots treated with aldrin and parathion at the rate of 5 pounds of toxicant per acre for control of the sugar-beet root aphid.

LOCATION	YEAR	TREATMENT	NO. OF BEETS AFTER THINNING (100-ROW FEET)	YIELD (TONS/ACRE)	MEAN INFESTATION RATING	
					During Summer	At Harvest
Monarch	1958	Aldrin	80	9.24	1.30*	2.20
		Parathion	79	8.88	1.44*	1.75
		Untreated	77	8.22	0.88	1.72
	1959	Aldrin	105	9.04	—	1.73*
		Parathion	105	9.94	—	0.95*
		Untreated	103	9.62	—	0.59
Coaldale	1958	Aldrin	91	22.10*	0.79*	1.36*
		Parathion	90	22.70*	0.68*	1.31*
		Untreated	86	19.00	1.30	0.90
	1959	Aldrin	108	18.70*	—	0.63
		Parathion	106	18.50*	—	0.47
		Untreated	108	17.44	—	0.56

* Significantly different from the untreated at the 5% level.

At Monarch in 1958 the infestation of aphids on July 30 was significantly higher on the insecticide-treated plots than on the untreated plots whereas at harvest there was no significant difference. In 1959 both the infestation rating and the percentage of beets infested at harvest were significantly higher on the treated plots than on the untreated ones. At Coaldale in 1958 the aphid infestation on August 4 was significantly higher on the untreated plots than on the insecticide-treated ones but at harvest the infestation was lower on the untreated plots (table 2). In 1959 the infestations on all the plots at Coaldale were low at harvest and there was no significant difference between untreated and insecticide-treated plots. The replicates were significantly different in infestation ratings, which indicated that the infestation was not uniformly distributed throughout the field. No other insect pest caused observable damage to the plots in either year.

There were no significant differences among the treatments in percentage sugar in either year at either location.

In both 1958 and 1959 the yields of beets at Coaldale were significantly higher than at Monarch. These higher yields appeared to be due mainly to moisture, as the Coaldale plots were irrigated more frequently than those at Monarch. At both locations the plots were irrigated by the farmers when they irrigated their own beets. The yields of beets in the insecticide-treated plots were higher than in the check plots in both years at Coaldale (table 2). There was, however, no significant difference in yields at Monarch, where moisture was limiting. The increase in yield at Coaldale in both years appeared to result from aphid control early in the season.

In these field experiments yield appeared to be primarily influenced by moisture and to a lesser extent by the control of the aphid with insecticides. At Coaldale, where moisture was adequate, yield was significantly increased by the application of insecticides whereas at Monarch, where moisture was limiting the insecticides had little effect on yield.

The mean numbers of *T. glabra* in the two-beet sample from each plot at Monarch in 1959 were:

Treatment		
Aldrin	*Parathion*	*Check*
60	85	316

In the fall there were significantly greater numbers of the predatory in the untreated plots than in the treated ones. The numbers of the predator ranged from 19 to 179 in the treated plots and from 75 to 559 in the untreated ones. In England large numbers of predators of the lettuce root aphid were present also in late summer. Dunn (1960) took counts of the aphids on lettuce at the end of August and found that predators had affected the relative aphid populations so that information could not be obtained at that time on control of the aphid with insecticides.

These results indicate that, although aldrin and parathion controlled the sugar-beet root aphid in experiments in the greenhouse, they did not control it throughout the season in fields of sugar beets. In the field, the insecticides reduced the populations of the aphid and, also, of the predator *T. glabra;* the aphids then were able to reinfest the beets without the restraining influence of predators. Thus, in the fall the aphid populations in untreated plots were the same as or lower than those in the insecticide-treated plots. At Coaldale, however, there was an increase in yield in both years that appeared to be due to control of the aphid early in the season. At Monarch, where moisture was a limiting factor, there was no increase in yield.

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Field Tests with New Insecticides for Control of the Sugarcane Borer in Louisiana in 1959[1]

S. D. HENSLEY,[2] W. J. MCCORMICK,[2] W. H. LONG,[3] and E. J. CONCIENNE[3,4]

ABSTRACT

In tests conducted in Louisiana in 1959, good control of the sugarcane borer, *Diatraea saccharalis* (F.), was obtained with granular formulations of 2% endrin and 2% S.D. 4402(1,3,4,5,6,7,8,8-octachloro-3*a*,4,7,7*a*-tetrahydro-4,7-methanophthalan). Granular formulations of 5% Guthion® (*O,O*-dimethyl *S*-(4-oxo-1,2,3,-benzotriazin-3-(4H)-ylmethyl) phosphorodithoate) and 10% Sevin® (1-naphthyl *N*-methylcarbamate) also showed some promise of being effective against the borer.

The sugarcane borer, *Diatraea saccharalis* (F.), has been responsible for an average annual loss to Louisiana cane growers of about 13% of their sugar yields since 1937 (Long et al. 1959).

Parasites and predators have failed to provide satisfactory control of the sugarcane borer. Jaynes & Bynum (1941) showed that laboratory rearing and release of native *Trichogramma* species were of no benefit in sugarcane borer control. As Clausen (1956) indicates, attempts to control this pest with exotic parasites introduced from tropical countries have not been successful. Newsom (1959) pointed out that none of the methods of biological control attempted in Louisiana in past years has prevented the sugarcane borer from causing severe crop losses.

Sodium fluosilicate, cryolite, and ryania have been recommended and used for sugarcane borer control since the early '20's. While these chemicals provided some control, they were much less effective than endrin which was first recommended in 1958 (Long et al. 1958). This recommendation provided for the broadcast application of 2% endrin granules at 14-day intervals at the rate of 12 pounds per acre per application for control of second- and third-generation infestations. As many as four applications are often applied.

In an effort to find additional insecticides for controlling the sugarcane borer, small-plot field tests with new insecticides are being conducted each year. Results from two tests conducted in 1959, one located at Southdown Plantation near Houma, La., and the other at Sterling Plantation near Franklin, La., are considered in this paper.

METHODS OF PROCEDURE.—A randomized complete block experimental design was employed in both tests. Treatments were replicated six times at Southdown and seven times at Sterling. Plots were three rows wide and 24 feet long (1/100 acre). The sugarcane variety C. P. 44–101 was treated at both locations. Treatments consisted of an untreated check and four biweekly applications of each of several insecticides starting June 30 for control of second- and third-generation infestations. Applications were begun after many second-generation eggs had hatched and the percentages of plants infested with young larvae which had not yet bored into stalks were, respectively, 12% at Southdown and 30% at Sterling Plantation.

The spray formulations were applied with a knapsack sprayer equipped with a fan-type nozzle. All applications of granulated insecticides in the Southdown test and the first two in the Sterling test were made with an electrical vibrator-type granule applicator developed by C. F. Henderson (unpublished paper). Long rods attached to each end of the applicator trough were used to raise it above the top of the tall cane. The insecticides were

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[2] Entomology Research Division, Agricultural Research Service, United States Department of Agriculture.

[3] Department of Entomology, Louisiana State University, Baton Rouge.

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Table 1.—Sugar yields and joints bored from small plots treated with granular insecticides against sugarcane borer, 1959. (Average of six replicates.)

INSECTICIDE	DOSAGE (LB./ ACRE)	JOINTS BORED (%)[a]	YIELD INCREASE (LB./ACRE)[a]
Southdown Plantation			
S. D. 4402, 2%	14.4	3 A	936 A
Guthion, 5%	18.4	9 B	966 A
Endrin, 2%	11.2	3 A	885 AB
Sevin, 10%	16.3	12 B	731 AB
Bayer 22408, 2.5%	18.1	19 C	529 ABC
Methyl Trithion, 5%	15.1	17 C	274 BC
Untreated[b]	—	30 D	— C
Sterling Plantation			
S. D. 4402, 2%	15.6	6 A	1,579 A
Endrin, 2%	13.4	9 AB	1,412 A
Guthion, 5%	20.6	14 B	1,389 AB
Sevin, 10%	19.7	20 C	1,345 AB
Methyl Trithion, 5%	23.8	36 D	972 AB
Dimethoate E.C. (4 lb./gal.)[c]	2.3	35 D	868 B
Bayer 22408, 2.5%	23.3	41 D	840 B
Bacillus thuringiensis W. P. (8×10^9) Spores/gram[c]	4.6	49 E	163 C
Untreated[b]	—	50 E	— C

[a] Means not followed by the same capital letter are significantly different from each other at the 5% level of probability according to Duncan's multiple range test.

[b] Actual yields from untreated plots at Southdown Plantation and Sterling Plantation, respectively, were 4,864 and 3,722 pounds of sugar per acre.

[c] Applied as a spray, dimethoate in 86.6 gallons of water and *B. thuringiensis* in 75.6 gallons.

applied in a 3-foot band over the row as two workers carrying the applicator walked down the row. The final two applications in the Sterling test were applied from a quart-size fruit jar equipped with a perforated metal top. This jar was inverted and fastened at an angle to one end of a long pole so that the insecticide could be sprinkled over the row from above the cane tops while the worker walked between rows.

The effect of treatments on sugarcane borer populations was estimated by determining the percentages of joints (internodes) showing external signs of borer tunneling at the time of harvest. Twenty stalks were examined from each plot.

All millable cane in each plot was hand cut and weighed. Sucrose content was determined by analysis of the juice extracted from the same 20 stalks from each plot that were examined for borer damage. Yield, expressed as sugar per acre, was estimated by multiplying the per cent of sucrose by the weight of the cane in each plot.

RESULTS.—The rates of insecticide applications and a summary of the results obtained in both tests are presented in table 1. In the Southdown Plantation test, all insecticide treatments caused significant reductions in borer damage. S. D. 4402 (1,3,4,5,6,7,8,8-octachloro-3*a*, 4,7,7*a*-tetrahydro-4,7-methanophthalan) and endrin were particularly effective and provided significantly better control than other insecticide treatments. Guthion® (*O,O*-dimethyl *S*-(4-oxo-1,2,3,-benzotriazin-3(4H)-ylmethyl) phosphorodithoate) and Sevin® (1-naphthyl *N*-methylcarbamate) gave significantly better control than that obtained with Methyl Trithion® (*O,O*-dimethyl *S*-(*p*-chlorophenylthio)methyl phosphorodithioate) or Bayer 22408 (*O,O*-diethyl *O*-naphthalimido phosphorothioate) which were significantly better than the untreated check. Increases in yield following treatment with insecticides ranged from 274 pounds of sugar per acre in Methyl Trithion-treated plots to 966 pounds per acre in Guthion-treated plots. Yields obtained from treatment with Guthion, S. D. 4402, endrin, Sevin, and Bayer 22408 did not differ significantly at the 5% level. Yields from plots treated with Methyl Trithion or with Bayer 22408 did not differ significantly from that measured in untreated plots.

In the test at Sterling Plantation, S. D. 4402, endrin, and Guthion provided significantly better control of borers than other insecticides and S. D. 4402 was significantly better than Guthion in this respect. Treatment with Sevin resulted in significantly better control than that obtained with Methyl Trithion, dimethoate, or Bayer 22408. *Bacillus thuringiensis* (thuricide) provided no control. Increases in yield following treatments ranged from 163 pounds of sugar per acre in *B. thuringiensis*-treated plots to 1,579 pounds per acre in S. D. 4402 plots. All plots except those treated with *B. thuringiensis* yielded significantly more sugar per acre than the untreated check. Yields from plots treated with S. D. 4402, endrin, Guthion, Sevin, and Methyl Trithion did not differ significantly at the 5% level and ranged from high to low in the order named. Only S. D. 4402 and endrin gave significantly better yields of sugar per acre than dimethoate or Bayer 22408.

DISCUSSION.—The percentages of joints injured in untreated plots show that insecticides were applied against much heavier borer infestations at Sterling Plantation than at Southdown. In both tests, S. D. 4402, endrin, Guthion, and Sevin provided effective borer control accompanied by large increases in yields of sugar.

Results from a test conducted in 1958 (Long et al. 1959) show that four biweekly applications of 5% granulated Sevin applied at a rate of 12 pounds per acre per application failed to provide satisfactory control of the sugarcane borer. In these tests, Sevin gave effective control when applied as a 10% granulated formulation at 16.3 and 19.7 pounds per acre. The control obtained with Guthion was slightly superior to that obtained with Sevin in the test at Sterling; however, the effectiveness of both insecticides against the borer should be studied further.

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Field Tests with New Insecticides for Sugarcane Borer Control in Louisiana in 1960[1]

W. H. Long,[2] S. D. Hensley,[3] E. J. Concienne,[2] and W. J. McCormick[3]

Abstract

In field experiments with new insecticides for control of the sugarcane borer, *Diatraea saccharalis* (F.), conducted in Louisiana during 1960, granular formulations of EPN and *Bacillus thuringiensis* Berliner showed sufficient promise to justify further work. Bayer 29493 (*O,O*-dimethyl *O*-[4-(methylthio)-*m*-tolyl] phosphorothioate), Nematocide 18133 (*O,O*-diethyl *O*-2-pyrazinyl phosphorothioate), phorate, and dimethoate showed little or no promise as insecticides for borer control.

Endrin has given good control of the sugarcane borer, *Diatraea saccharalis* (F.), since it was recommended for use on sugar cane in 1958. However, field tests have been conducted annually to find other insecticides for borer control. This paper reports results from two such tests conducted during 1960 at Donaldsonville and Franklin, Louisiana, respectively.

Methods.—All insecticides were in granular formulations on clay carriers and were applied in the amounts and concentrations indicated in tables 1 and 2. Insecticides were applied to 1/100-acre plots (3 rows×24 feet) using hand equipment. At Donaldsonville a specially designed granule applicator 6 feet wide was carried through the plots by two men to attain broadcast application. At Franklin the insecticide granules were sprinkled in a band over the row from glass fruit jars with perforated caps. Insecticides were applied July 7, July 19, August 4, and August 17 at Donaldsonville, and July 20, August 4, and August 17 at Franklin.

Borer infestations and crop yields were determined in the fall at harvest. Estimates of infestation were made by determining the percentages of internodes with external signs of borer tunneling on 20 randomly selected stalks from each plot. Infestations then were expressed as percentages of joints bored. All of the cane in each plot was weighed and the average numbers of tons of net cane per acre were computed for each treatment. Net cane was determined by deducting 10% for dead leaves and trash from the gross weight of the cane in one experiment, and by burning the cane before weighing it in another.

Yields in pounds of sugar per ton of cane were estimated by polarimetric determinations of the percentages of sucrose in the juice from each 20-stalk sample examined for borer infestation. Sucrose analyses of the juice were then used to compute pounds of sugar per ton of cane by the Winter-Carp-Geerlings formula in the experiment at Donaldsonville, and by empirical data on commercially recoverable sugar from Louisiana sugar cane in the experiment at Franklin (Anon. 1959).

Randomized complete block designs were used in both experiments with each treatment replicated six times. Analyses of variance were made with the data, and Duncan's Multiple Range Test was applied to each set of means as recommended by LeClerg (1957).

Table 1.—Small-plot experiment with granular insecticides applied broadcast for sugarcane borer control, Salsburg Plantation, Donaldsonville, Louisiana, 1960.[a]

Insecticides	Avg. Rate per Application (lb./acre)	Joints Bored (%)	Yield per Acre	
			Tons of Cane	Sugar (lb.)
Endrin, 2%	12	6 A	27.4	5,380 A
EPN, 1%	26	19 B	25.8	5,168 A
EPN, 1%	13	23 B	23.2	4,489 BC
B. thuringiensis[b]	75	21 B	25.5	5,081 AB
Bayer 29493, 5%	12	28 C	22.8	4,523 BC
Phorate, 10%	13	32 C	23.1	4,379 C
Dimethoate, 10%	12	29 C	21.8	4,226 C
Untreated		31 C	21.9	4,381 C

[a] Means not followed by the same capital letter differ significantly at the 5% level of probability.

[b] A granular formulation containing 3×10^9 bacterial spores per gram furnished by the Bioferm Corporation, Wasco, California.

Table 2.—Small-plot experiment with band applications of granulated insecticides for sugar cane borer control, Sterling Plantation, Franklin, Louisiana, 1960.[a]

Insecticides	Avg. Rate per Application (lb./acre)	Joints Bored (%)	Yield per Acre	
			Tons of Cane	Sugar (lb.)
Endrin, 2%	23	15 A	17.0	3,789 A
EPN, 1%	19	34 B	17.6	3,886 A
B. thuringiensis[b]	20	31 B	16.8	3,673 AB
Nematocide 18133, 10%	13	36 BC	16.5	3,469 AB
Bayer 29493, 5%	57	42 BC	17.1	3,438 AB
Phorate, 10%	22	57 D	16.3	3,214 AB
Dimethoate, 10%	15	60 D	16.4	3,070 B
Untreated		48 CD	14.7	3,080 B

[a] See footnote a, table 1.

[b] See footnote b, table 1.

Results.—Table 1 shows that treatment with endrin resulted in fewer joints bored than any other insecticide in the test at Donaldsonville. While EPN and *Bacillus thuringiensis* Berliner were inferior to endrin, they gave significantly better borer control than any of the remaining insecticides used in this test. There was no significant difference in per cent of joints bored between the untreated check plots and plots treated with Bayer 29493 (*O,O*-dimethyl *O*-[4-(methylthio)-*m*-tolyl] phosphorothioate), phorate, and dimethoate (table 1).

Table 2 shows that endrin also reduced the percentage of joints bored significantly more than any other insecticide in the test at Franklin. EPN, *B. thuringiensis*, Nematocide 18133 (*O,O*-diethyl *O*-2-pyrazinyl phosphorothioate), and Bayer 29493 were inferior to endrin in

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[2] Department of Entomology Research, Louisiana Agricultural Experiment Station, Louisiana State University, Baton Rouge.

[3] Entomology Research Division, Agricultural Research Service, U. S. Department of Agriculture.

borer control, but were significantly better than phorate or dimethoate. Nematocide 18133, Bayer 29493, phorate, and dimethoate did not differ significantly in per cent of joints bored from the untreated check.

The largest yield increases in the test at Donaldsonville resulted from treatments with endrin and EPN (table 1). Twelve pounds of 2% endrin and 26 pounds of 1% EPN per acre per application resulted in significantly larger yields of sugar per acre than any other insecticide. *B. thuringiensis* gave the third highest yield of sugar which was significantly better than the untreated check, and not significantly different from yields obtained with endrin and the high rate of EPN. Yields of sugar following treatments with Bayer 29493, phorate, dimethoate and the low rate of EPN were not significantly different from the untreated check.

The best yields of sugar obtained in the test at Franklin resulted from treatments with endrin and EPN (table 2). These insecticides gave significantly better yields than dimethoate or the untreated check. Yields with *B. thuringiensis*, Nematocide 18133, Bayer 29493, and phorate did not differ significantly from the high yields gotten from endrin and EPN or the low yields from dimethoate and the untreated check.

DISCUSSION.—EPN and *B. thuringiensis* showed sufficient promise in these tests to deserve further study as possible control agents for the sugarcane borer.

B. thuringiensis was previously tested in the field against the sugarcane borer by Hensley et al. (1961) but without success. However, in this earlier test a wettable powder was used in spray treatments. Comparison of these older results with the ones reported in this paper leads us to conclude tentatively that granular formulations of *B. thuringiensis* may be much more effective than sprays for sugarcane borer control.

The data in tables 1 and 2 indicate that correlations between borer control, as determined by per cent joints bored, and increases in yields of cane or sugar per acre are not always as high as might be desired. This suggests a need for improvement of the present widely used method of measuring borer infestations in sugar cane by estimating the percentage of joints bored.

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Resistance of Sweetclover to the Sweetclover Aphid[1]

G. R. MANGLITZ and H. J. GORZ,[2] *Agric. Res. Serv., U.S.D.A., Lincoln, Nebraska*

ABSTRACT

An evaluation of resistance to the sweetclover aphid (*Therioaphis riehmi* (Börner)) was made among commonly grown sweetclover varieties and the more promising breeding lines. This evaluation, made both in the greenhouse and under field conditions, was based on aphid populations and on plant survival under heavy aphid infestations. A highly significant negative correlation was demonstrated to exist between aphid populations and plant survival. Of the plant materials evaluated, the breeding line N13 and the variety Spanish were the most resistant whereas common yellow was the most susceptible. Great variability existed both within and between the strains and varieties tested.

Plant age had a pronounced effect on resistance. The older plants demonstrated the greatest resistance among most of the materials tested. The least influence of plant age was shown by the most resistant (N 13) and the most susceptible (common yellow) entries.

The outlook for combating the sweetclover aphid through the development and use of aphid-resistant varieties appears promising.

The sweetclover aphid (*Therioaphis riehmi* (Börner)), now distributed over the entire United States, was first found in this country in 1948 (Dickson 1959). Its presence in the United States, however, was not generally recognized until intensive surveys were made on the distribution of another introduced legume pest, the spotted alfalfa aphid (*Therioaphis maculata* (Buckton)) (Peters & Painter 1957). Plant injury by the sweetclover aphid has not been so severe or consistent as the injury on alfalfa by the spotted alfalfa aphid, but economic damage does occur in the sweetclover-growing area of the Northern Great Plains (MacNary 1959, Peters & Painter 1958). Kieckhefer (1958) reported that the most serious damage by the sweetclover aphid was in association with injury by the sweetclover weevil (*Sitona cylindricollis* Fåhraeus).

The host range of the aphids of the genus *Therioaphis* occurring in this country has been studied in detail by Peters & Painter (1957, 1958). These authors reported that the host range of the sweetclover aphid was restricted to two closely related plant genera, *Melilotus* and *Trigonella*. They also reported that within a plant species considerable variation in reaction to the aphid may occur. Preliminary investigations that were the forerunner of the present study (Howe & Manglitz 1959) indicated encouraging results in finding plants resistant to the sweetclover aphid.

The objectives of the present study were to evaluate the reaction of commonly grown varieties and breeding

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[2] Entomologist, Entomology Research Division and Geneticist, Crops Research Division, respectively. In cooperation with the Departments of Entomology and Agronomy, University of Nebraska.

lines of sweetclover to the aphid and to determine the effects of plant age on reaction to the aphid. The investigations reported are a part of a larger study, which has the ultimate objective of finding a high level of plant resistance that can be incorporated into improved varieties of sweetclover.

MATERIALS AND METHODS.—Plant material for initial evaluations consisted of commonly grown varieties and improved strains of white-flowered sweetclover (*Melilotus alba* Desr.) and yellow-flowered sweetclover (*M. officinalis* (L.) Lam.). Progenies of selected plants were grown from seed produced in the greenhouse. Characteristics of the plant materials are shown in table 1. Among the agronomic characteristics mentioned in this table, low coumarin content and large seed size are both highly desirable. Most of the sweetclover acreage in the North-Central States is seeded to varieties that are biennial in growth habit.

A greenhouse colony was started from aphids collected in the field in Lancaster County, Nebraska. The aphids were cultured on common yellow sweetclover and were grown under a daily photoperiod of 16 to 18 hours.

Greenhouse tests, conducted from late fall to early spring during 1958–59 and 1959–60, were of two general types. The first consisted of caging a given number of aphid nymphs on a portion of large, potted plants and observing survival and subsequent reproduction. The other type of test involved mass infestations of small seedling plants with subsequent observations on plant survival. In the latter tests, entries were planted in rows in greenhouse flats at the rate of 20 to 30 seeds per row with 12 rows per flat. Entries in these tests were both randomized and replicated.

Field evaluations of resistance and the effect of plant age on resistance were made in 1959 and 1960. Plant-age studies were conducted in a 12- × 12-foot walk-in, screened cage during the summer of 1959. Plantings of six varieties were made on three different dates in a randomized, complete-block design with four replications. Plantings for studies of resistance were made in 1960. In the spring, two varieties and two lines were planted in a screened cage in a randomized, complete-block design with four replications. Two field tests, conducted without cages, included five varieties, two lines, and one selection. In one test, three replications of the sweetclover entries were seeded in the spring, whereas in the other test, a fall seeding with four replications was used. Rows were spaced 18 inches apart in the caged plots and 24 inches apart in the uncaged plots. Plant-mortality observations were based on initial and final plant-stand counts. Aphid populations were sampled by one of two methods, a stem count or a leaf count. With the leaf count, it was possible to place the leaves of a single sample in a petri dish and freeze them. This procedure permitted the sampling of a field experiment to be completed in a single day whereas careful counting could be continued over a much longer period. Freezing was also superior to other methods of preservation in that aphid appearance remained natural so that different species and diseased and parasitized individuals could be easily detected. The aphids remained in place on the leaves, which made accurate counting easier than if the aphids were matted together in a killing solution.

Table 1.—Plant materials used for evaluating resistance of sweetclover to the sweetclover aphid, 1958–60.

PLANT DESIGNATION[a]	SPECIES OF *Melilotus*[b]	REMARKS
Variety		
Common white	*alba*	
Cumino	*alba*	Improved var. from Can.
Evergreen	*alba*	[c]
Spanish	*alba*	[c]
Floranna	*alba annua*	
Hubam	*alba annua*	
Israel	*alba annua*	
Erector	*officinalis*	
Goldtop	*officinalis*	Improved var. from Wis.[c]
Madrid	*officinalis*	[c]
Common yellow	*officinalis*	Most commonly grown var. in Nebr.
Golden Annual	*suaveolens annua*	
Line or experimental variety		
W 7	*alba*	Improved line from Wis.
W 31	*alba*	Improved line from Wis.
N 13	*officinalis*	Large-seeded line from Nebr.
Selection		
G 306	*officinalis*	From Madrid
G 314	*alba*	From Spanish
G 315	*officinalis*	From N 13
G 328	*officinalis*	From N 13

[a] All plants with high coumarin content except Cumino and W 31.
[b] All species biennial except those designated *annua*.
[c] Designates variety recommended for planting in all or part of Nebraska.

RESULTS.—*Greenhouse Variety Studies.*—Results of caging aphids on mature plants of different varieties indicated great variability in aphid reproduction. The mean numbers of aphids produced by three individuals in a 17-day period varied from 13 for Spanish to 64 for common yellow, but the within-variety variation prevented statistical significance. Thus it seemed apparent that a large sample of plants must be examined for evaluation of aphid resistance among the varieties.

A preliminary test with mass infestation of 2-week-old seedlings indicated that their survival under conditions of heavy infestation would be a means of detecting resistance. A more detailed mass-infestation test was then conducted with 3 replications of seedlings of 11 sweetclover varieties and 1 of Ladino white clover (*Trifolium repens* L.), a nonhost of the aphid. The following percentages of seedling survival were obtained: Ladino white clover, 91.9; N 13, 77.7; Spanish, 58.0; Madrid, 53.2; W 7, 45.6; Cumino, 44.7; W 31, 37.6; Israel, 35.5; Hubam, 35.0; Goldtop, 33.0; common white, 29.5, and common yellow, 13.6. These mean percentages of survival were significant at the 1% level.

In a second greenhouse test, in which 16 entries were replicated 4 times, plant survival was ascertained on the total plant population and aphid counts were made on 5 seedlings of each entry 2 times after infestation. The results of this test are presented in table 2. N 13 and Spanish had the highest rates of survival among the entries that were included in both tests. Common yellow had the lowest survival in the first test and was one of the lowest in the second test. As seen in table 2, the highest means of plant survival in the second test were obtained with G 315, N 13, and G 306. The means for these entries are not significantly different from each other but are, as a group, significantly different from means that are equivalent to or less than the percentage obtained for Goldtop. The remaining means are intermediate to both groups and although statistically overlapping in each direction, they overlap to a greater extent into the group with the lower mean survival rates.

Table 2.—Performance of eleven varieties, two breeding lines, and three selections in the second greenhouse sweetclover variety test in which seedling plants were subjected to heavy sweetclover aphid infestations. Fall 1959.

Entry	Plant Survival[a] (%)		Entry	Aphids per 5 Plants on— Oct. 22[b]		Oct. 29[b]
Israel	0	d	Goldtop	121.8	a	120.3
Common yellow	4.1	d	Hubam	106.8	ab	125.3
Cumino	13.9	cd	Erector	98.8	abc	44.5
Erector	14.1	cd	Common Yellow	96.5	abc	71.3
W 31	16.3	cd	Cumino	91.3	abc	34.3
Madrid	18.5	cd	W 31	87.0	abcd	75.3
Hubam	21.1	cd	Israel	79.5	abcd	26.3
G 314	23.5	cd	Madrid	77.8	abcd	50.0
Golden Annual	24.9	cd	Floranna	73.5	abcd	99.3
Goldtop	27.1	cd	Golden Annual	64.5	bcde	108.3
Floranna	36.2	bc	N 31	62.3	bcde	34.5
Evergreen	36.4	bc	Evergreen	55.8	cdef	52.8
Spanish	37.6	bc	G 314	55.8	cdef	41.8
G 306	57.6	ab	Spanish	39.3	def	53.5
N 13	71.5	a	G 306	18.8	ef	18.0
G 315	79.6	a	G 315	5.0	f	14.3

[a] Means not followed by the same letters are significant at the 5% level. Means followed by the same letters are not significantly different.
[b] Means are statistically significant but mean separations not shown.

The entries with the highest percentages of plant survival present a variable picture with regard to aphid populations. The separation of the entries into statistically significant groups on the basis of the mean number of aphids at the first counting date is not as clear-cut as with the mean survival rates. G 315, which is the S_1 progeny of a single resistant plant, averaged only one aphid per plant at the first counting date. The experimental variety, N 13, contains a majority of highly resistant plants, but is actually heterozygous, since intermediate and susceptible plants may often appear. One or two highly susceptible plants in one replication of N 13 were largely responsible for the high average counts found in this line. The variety Goldtop supported the greatest number of aphids but was intermediate in survival rate. Since the means for Goldtop were not significantly different from those of the more susceptible varieties for both plant survival and aphid counts, it may be concluded that this variety is not tolerant to aphid attack.

The second aphid count, made on October 29, does not reflect the relative resistance for all entries. Those with the lowest survival ratings were so heavily damaged at the time of the second counting that they could no longer support heavy populations of aphids. Little change was noted in the numbers of aphids on the entries with intermediate and higher rates of survival. The percentage of plant survival and number of aphids at the first counting date were found to be negatively correlated with a highly significant coefficient of -0.727. The second aphid count and plant survival were not significantly correlated ($r = -0.325$).

Field Variety Studies.—In the test with the spring-planted varieties, two aphid counts on a stem basis were made at times when the population was increasing. Aphids on 10 stems were counted at the early date and those on five stems at the later date. In table 3 results are shown as the number of aphids per five stems. The means on the second sampling date (counts made on five stems per entry) are not significantly different from each other but are ranked in essentially the same order as those obtained on the first date. They are also in essentially the same order as they were ranked in greenhouse studies. The aphid populations suddenly dropped in magnitude and plant damage did not reach the point at which reliable observations could be made.

Table 3.—Numbers of aphids on uncaged sweetclover seedlings field-planted on April 15, 1960.

Entry	Aphids per 5 Stems on— June 24[a]		July 27[b]	Percentage of Performance of Common Yellow on— June 24	July 27
G 328	0.3	a	11.0	1.8	1.8
N 13	1.8	ab	72.7	10.9	17.4
W 31	5.8	ab	39.3	35.2	6.3
Spanish	9.1	abc	262.7	55.2	41.9
Evergreen	9.5	abc	494.7	57.6	79.0
Madrid	9.8	abc	461.0	59.4	73.6
Goldtop	11.6	bc	346.0	70.3	55.3
Common yellow	16.5	c	626.0	100.0	100.0

[a] Means not followed by the same letters are significantly different at the 5% level. Means followed by the same letter are not significantly different.
[b] Means not statistically significant.

Counts made in the caged-variety planting in August were based on the aphids on 100 trifoliolate leaves per entry. The results, shown as mean numbers per 50 leaves for easy comparison with uncaged plantings, were: N 13, 36.9; W 31, 130.4; Spanish, 148.4 and Goldtop, 176.9. These means are significantly different at the 5% level. The aphid populations on N 13, W 31, and Spanish were, respectively, 20.8%, 73.7% and 83.9% of the population on Goldtop. Also the percentage of adults, when compared with the total population, was greater on N 13 (9.9%) than on W 31, Spanish, or Goldtop (8.2%, 8.1% and 8.5%, respectively). The higher percentage of adults on N 13, which had the smallest population of aphids, probably indicated a lower rate of reproduction on this strain of sweetclover.

On October 10 counts were made on 50 leaves per entry in the fall-planted variety test. Results are shown in table 4. The means for the numbers of sweetclover aphids were significantly different at the 1% level. With the striking exception of common yellow, the entries were in essentially the same order as in the spring test. The percentages of adults were similar to those obtained in the caged-plot test. The selection G 328, although not included in the caged-plot test, was the most resistant entry in the fall-planted test and had the highest percentage of adults, which presumably indicated a lower reproductive rate by

Table 4.—Numbers of sweetclover aphids on young uncaged sweetclover seedlings planted out-of-doors on August 23; counts made October 10, 1960.

Entry	Aphids per 50 Trifoliolates Number[a]		Percentage of Goldtop	Adults (%)
G 328	154.00	a	24.1	11.2
N 13	207.75	ab	32.5	8.9
Common yellow	284.75	abc	44.5	8.5
W 31	386.25	bcd	60.4	8.7
Spanish	400.75	cd	62.6	8.6
Madrid	401.50	cd	62.7	8.6
Evergreen	524.50	de	81.9	9.7
Goldtop	640.00	e	100.0	7.7

[a] Means not followed by the same letters are significantly different at the 5% level. Means followed by the same letters are not significantly different.

aphids on this entry. Typical sweetclover aphid injury began appearing in late fall on the most heavily infested varieties, but frost injury complicated the symptoms so that accurate observations could not be made.

Observations were made in all of the field experiments to detect factors other than host-plant resistance that might influence sweetclover aphid populations. Predators were not a factor under the screen cages and were held in check, when necessary, in the uncaged experiments by occasional applications of methoxychlor. Light populations of a parasite (*Trioxys ulitis* Mues.) and small numbers of aphids killed by an entomophagous fungus were found in several of the experiments but were not sufficient to mask or upset the influence of host-plant resistance.

Plant-Age Studies.—Seed of six varieties was planted in the cage on three different dates, spaced 18 days apart. Eighteen days after the last planting, a stand count was made and the aphid infestation was allowed to build up in the cage, which at that time contained plants 18, 36, and 54 days of age. A final stand count was made late in October and plant survival calculated. In the majority of the entries, the older seedlings had the highest survival rate. The percentage of plant survival and counts of aphid populations are presented in table 5. None of the entries with a mean survival percentage higher than that of Israel (aged 36 days) were significantly different from each other, but this resistant group was significantly different from the entries with a mean survival percentage lower than that of Israel. As can be seen, plant survival for Israel (aged 36 days) overlaps statistically into each of the other two groups. In the group with the highest percentage of survival, the only 18-day-old entry was N 13, which was consistently the most resistant entry tested. In the group with the lowest percentage of survival, the only entry that was 54 days of age was common yellow, which is highly susceptible to the aphid. Visual plant injury ratings were made twice and again the older seedlings of each variety or line showed the least injury. Aphid counts were made on three dates from late August to early October. Based on the number of aphids per 20 trifoliolate leaves, these counts gave a better estimate of aphid density than total population per plant because they were not biased by the great variation in number of leaves per plant. The first count, results of which are presented in table 5, was the only one in which all entries were observed. At the later dates, plant defoliation had progressed to the point at which counts were not possible on the more susceptible plants. Plant survival and aphid density were found to be negatively correlated with a highly significant coefficient of -0.757.

Figure 1 shows the plotting of plant survival versus plant age under heavy sweetclover aphid infestation. A general trend toward increased survival with increased plant age at time of infestation can be observed. The least increase in survival with an increase in age is noted in the most resistant (N 13) and most susceptible entries (common yellow) whereas the greatest increase is seen in the intermediate entries. An examination of table 5 shows that no significant difference existed between the means for plant survival for the three ages of N 13. In all of the remaining entries, except common yellow, distinct differences in mean plant survival existed between two or more of the age groups. Differences in survival of common yellow are not statistically clear-cut. When studying the effect of plant age on the survival of alfalfa under heavy spotted alfalfa aphid infestations, Howe & Pesho (1960) also found higher survival in older plants.

Table 5.—The effect of varietal difference and plant age when infested, on sweetclover survival under aphid attack. Outdoor cage. 1959.[a]

Entry	Plant Age (days)	Plant Survival (%)[b]		Entry	Plant Age (days)	Aphids per 20 Trifoliolates[b]	
Common yellow	36	5.4	ef	Common yellow	36	114.2	a
Common yellow	18	9.9	ef	Common yellow	18	106.0	ab
Cumino	18	16.0	def	Israel	18	84.0	abc
Madrid	18	18.6	def	Israel	36	80.0	bc
Israel	18	24.4	def	Cumino	36	67.0	cd
Common yellow	54	37.8	cde	Spanish	36	59.0	cde
Cumino	36	38.2	cde	Madrid	18	52.0	cdef
Spanish	18	38.6	cde	Cumino	18	50.0	cdef
Madrid	36	42.2	cd	Madrid	36	48.7	cdef
Israel	36	54.8	bc	Common yellow	54	34.5	def
N 13	18	73.7	ab	Israel	54	34.0	defg
Madrid	54	73.9	ab	Spanish	18	27.2	defg
N 13	36	80.1	ab	Madrid	54	27.0	defg
Spanish	36	82.6	ab	N 13	18	21.0	efg
Cumino	54	85.1	ab	Cumino	54	15.5	fg
Israel	54	93.1	a	N 13	36	3.0	g
Spanish	54	96.8	a	Spanish	54	2.0	g
N 13	54	98.9	a	N 13	54	1.0	g

[a] The aphid population was controlled with an insecticide until seedlings reached the desired age. A natural infestation was then allowed to build up.

[b] Means not followed by the same letters are statistically significant at the 5% level. Means followed by the same letters are not significantly different.

DISCUSSION.—A rating of the different plant materials tested could be clearly made only for the more resistant (N 13 and Spanish) and the more susceptible (common yellow) varieties. The intermediate types remained intermediate in all tests but not always in the same order.

Close agreement was shown between ratings made in the greenhouse and those made under field conditions except for common yellow in the field test in the fall of 1960. In this test, common yellow supported low populations of aphids, but in the greenhouse and in the remainder of the field tests, populations were high. It is

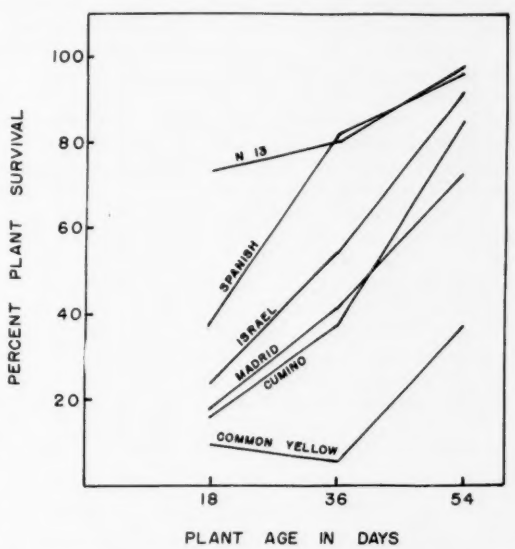

FIG. 1.—The effect of plant age at the time of infestation on plant survival under heavy aphid infestation in a field cage.

probable that lower prevailing temperatures influenced the size of the populations. An increase in aphid reproductive ability seemed to occur on the more resistant entries as evidenced by the fact that the population on the most resistant line (G 328) was only 1.8% of the population on the most susceptible entry in the June and July test, whereas in the fall test the population was 24.1% of that on the most susceptible entry. Plant age may also have been an important factor since the spring planting was 60 days old at the time of the first count but the fall planting was only 48 days old. However, the tendency toward greater aphid survival on resistant plants under lower temperatures was also noted for the spotted alfalfa aphid (Howe & Smith 1957).

The methods used in field sampling of aphid populations should be considered. The data presented in table 3 indicate that when 10 stems were used on the first sampling date (June 24), significant mean differences were shown to exist whereas when five stems were used on the second date (July 27), the mean differences were not significant. Changes were observed in the magnitude of the population, but a larger sample size was felt to be important, particularly in evaluating lines and varieties. Since wide differences in aphid reaction have been shown to exist between individual plants within a line, the use of a trifoliolate leaf count to observe an aphid population seems better than a stem count because the leaf permits sampling from a much larger plant population. The leaf also seems to be a better standard of comparison among varieties because less variation occurs in leaf size and shape than in stem size and shape. When Nielson (1957) compared six aphid sampling methods on alfalfa he also concluded that the leaf count was most practical and accurate.

The highly significant negative correlation between plant survival and aphid populations indicates that the resistance demonstrated resulted from antibiosis or nonpreference rather than plant tolerance. The terminology used here in describing the types of resistance was defined by Painter (1951). This correlation also shows the validity of rating plants for resistance on the basis of aphid populations alone.

The results of the studies on the effect of plant age on resistance demonstrate the importance of testing for this character in seedlings. They also emphasize the importance of getting plants safely past the seedling stage in the field. Resistance incorporated into a variety should be of a type that has maximum possible expression in the very young seedling.

With an insect such as the sweetclover aphid, which has a wide distribution range but does not build up to damaging numbers every year or throughout its range, the widespread use of resistant varieties could virtually eliminate damage by this insect. Its restricted host range, with almost none of its wild hosts present in the areas of economic damage, greatly favors this outlook. Much volunteer sweetclover does exist wherever this crop is grown, but the aphid populations that might develop on it would not be important if lines with high levels of resistance could be developed.

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Esterase Inhibition in Organophosphorus Poisoning of House Flies[1]

R. D. O'Brien,[2] *Department of Entomology, Cornell University, Ithaca, New York*

Abstract

The inhibition of hydrolysis of acetylcholine, phenyl acetate, tributyrin and methyl butyrate by house flies (*Musca domestica* L.) has been studied at various times after poisoning by four organophosphates: parathion, malathion, Diazinon® (*O,O*-diethyl *O*-2-isopropyl-4-methyl-6-pyrimidinyl phosphorothioate) and Co-ral® (*O,O*-diethyl *O*-3-chloro-4-methyl-2-oxo-2*H*-1-benzopyran-7-yl phosphorothioate). In surviving flies, cholinesterase was invariably the least inhibited enzyme. But in killed flies, cholinesterase was usually the most inhibited enzyme, being 75% to 99% inactivated. The difference between enzyme levels of killed and surviving flies at any time was also greatest for cholinesterase. It is concluded that death is associated with, and probably due to, cholinesterase inhibition.

The insecticidal action of organophosphates is generally attributed to inhibition of cholinesterase. Nevertheless, other esterases are inhibited in the course of poisoning, as first indicated by *in vitro* studies (Lord & Potter 1954), and later confirmed *in vivo* for the case of *o*-nitrophenyl acetate hydrolysis in the locust (Hopf 1954) and American cockroach (Casida 1955). The relative extent of *in vivo* inhibitions of cholinesterase and other esterases can only be evaluated when protective techniques are used, to avoid homogenization artifacts, i.e., bringing into contact, by homogenization, inhibitor and enzyme which were separated *in vivo*, thus obtaining erroneously high estimates of *in vivo* inhibition. The protective technique involves homogenizing in substrate, to protect against subsequent inhibition (van Asperen 1958).

With protective techniques, van Asperen (1960) showed in house flies poisoned by any of six organophosphates that at time of knockdown cholinesterase was inhibited by 27% to 51%, but methyl butyrate hydrolysis by 75% to 95%. Similar observations have been made on survivors from poisoning by Stegwee (1960) for TEPP and by Plapp & Bigley (1961) for parathion and malathion at various times after treatment.

The present study, conclusions of which were briefly reported earlier (O'Brien 1960) is substantially like that of Plapp and Bigley, but with additional organophosphates and more substrates, and including observations on the killed as well as the surviving house flies (*Musca domestica* L.). The reason for using several substrates is that house flies may have several aliesterases and at least one aromatic esterase (van Asperen 1959, Metcalf et al. 1956).

Methods.—Groups of 100 female house flies, 1 day old, were treated topically with acetone solutions of the insecticides, at the predetermined LD_{50} dose. At the indicated times, symptoms were noted, deaths counted, and the surviving and dead flies frozen separately in test tubes by immersing the tubes in a mixture of solid CO_2 and ethanol. A duplicate run was made several days later.

Within 24 hours, flies (3 per ml.) were homogenized in iced phosphate buffer, 0.067M, pH7.2, in an iced Potter-Elvejhem homogenizer for 1 minute. Duplicate 1-ml. portions were pipetted into iced Warburg flasks containing 1 ml. of either 2.2×10^{-2}M phenyl acetate with 2×10^{-5}M eserine, or 3.3×10^{-2}M acetylcholine bromide, or 1.1×10^{-2}M methyl butyrate, or a 2% emulsion of tributyrin in 1% Triton X-100. (Eserine was used in the one case because phenyl acetate is in part hydrolyzed by cholinesterase; this part of the hydrolysis is eliminated by eserine (van Asperen 1959)). Then 0.2 ml. of 1.25% $NaHCO_3$ was added, the flasks were attached to the manometers and placed in the bath (25° C.), gassed 5 minutes with 5% CO_2 in N_2, 5 minutes later the flasks were closed and measurements begun.

Phosphate buffer is used in this technique to avoid destruction of "aliesterase" due to alkaline conditions on adding bicarbonate. Retention occurs, i.e., not all the CO_2 produced is released. Its extent varies with the volume evolved, e.g., 10% at 100 μl., 25% at 300 μl. (calculated from Birmingham & Elliott 1952).

The above technique is a variation of the full protective technique, in which substrate is present during homogenization. The full technique would have required multiple homogenizations, because four substrates were under study. The homogenizations were brief and cold, and since the extent of reaction of organophosphates with esterases is time- and temperature-dependent, negligible inhibition could occur at this stage. The major risk of artifactual inhibition in unprotective techniques is in the Warburg flask prior to tipping in substrate. This possibility is avoided in the present technique by mixing enzyme with substrate immediately after homogenizing. These precautions should be more than adequate to prevent artifacts in view of the evidence that artifacts have only been observed with phosphates, never with phosphorothionates (Colhoun 1959, Seume et al. 1960, Plapp & Bigley 1961). All four compounds studied here were phosphorothionates.

Results.—The results for surviving house flies are presented in figure 1. They show clearly that for all of the organophosphates tested, cholinesterase was the least inhibited enzyme; its maximum inhibition was 72%, found with Co-Ral®(*O,O*-diethyl *O*-3-chloro-4-methyl-2-oxo-2*H*-1-benzopyran-7-yl phosphorothioate). The minimum was 50%, found with parathion. By contrast, hydrolysis of other substrates than acetylcholine was severely inhibited (80% to 100%) in every case. The scatter observed with results for inhibition of hydrolysis of methyl butyrate is due to the low values involved: the average of eight control values was 24 μl. of CO_2 per 30 minutes, so a scatter of 20% (the estimated maximum) means an error of 5 μl. By contrast, average control values for acetyl choline were 53 μl., for phenyl acetate 67 μl. and for tributyrin 118 μl.

Previous studies have examined only inhibition of

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cholinesterase and "aliesterase," defined as methyl butyrate hydrolysing enzyme(s). Yet there was evidence that there was more than one "aliesterase": for instance, methyl butyrate and tributyrin may be hydrolyzed by different enzymes. Phenyl acetate can be hydrolyzed by cholinesterase (van Asperen 1959) as well as by other esterases(s). In the present study eserine was used with the phenyl acetate to ensure that the cholinesterase-catalyzed reaction was suppressed. Whether the residual reaction was caused by the same enzyme that hydrolyzes methyl butyrate is uncertain (van Asperen 1959); an aromatic esterase has been reported in some insects, although it was absent in fly heads (Metcalf et al. 1956). The present study shows that whether or not methyl butyrate, triacetin and phenyl acetate hydrolyses are catalyzed by a single "aliesterase," they are all affected in the same way in *in vivo* by the four organophosphates used, when whole surviving flies are studied.

Some aspects of the results disagree with those of Plapp and Bigley, who found no "recovery" of house fly head cholinesterase after malathion, and almost none after parathion. In the present study, cholinesterase "recovery" was found for all compounds, but was never complete, in contrast to the findings of Mengle & Casida (1958) and Mengle & O'Brien (1960), who examined house fly head cholinesterase, but in agreement with the findings of O'Brien (1956) who used whole house flies as in the present study, and Stegwee (1960) who studied heads and thoraces separately.

Plapp and Bigley found that inhibition of methyl butyrate hydrolysis by an LD_{50} of malathion or parathion was very rapid, but returned to zero inhibition in about a day. In the present study inhibition of methyl butyrate hydrolysis was more severe (about 90%, compared with Plapp and Bigley's 50%) and "recovery" was only partial. Consequently, inhibition of "aliesterase" was always greater than that of cholinesterase.

The esterases in house flies killed by the LD_{50} of organophosphates were also examined, with the results shown in table 1. These data are from the same experiments as those described above. Sufficient dead flies were available only at the 320- and 1,280-minute intervals.

It has been pointed out (O'Brien 1960) that in experiments of this kind, one is sampling a shifting population. An important consequence has been derived by Van Asperen (private communication), who shows that such data do not prove that true recovery occurs. For instance if 50% of the flies had an esterase severely inhibited, and were all dead by 80 minutes, while the other 50% had that esterase mildly inhibited and were not killed at all, and if no true esterase recovery occurred, one would find apparent rapid recovery of esterase activity after 80 minutes when only living flies are used. If instead one assumes a normal distribution of susceptibilities to esterase inhibition and to lethal action, and assumes that mortalities start at about 80 minutes and that those with most esterase inhibition die first, one would get curves very like those observed, even though no true recovery occurred. Furthermore one would get apparent recovery of esterase in dead flies, because those taken at 320 minutes would include only those with most-inhibited esterases, whereas those at 1,280 minutes would also include those with less-inhibited esterases.

Table 1.—Level of esterase activity in dead houes flies, as per cent of normal.

Substrate: Time After Treatment (min.):	Acetyl-choline		Phenyl Acetate		Methyl Butyrate		Tributy-rin	
	320	1280	320	1280	320	1280	320	1280
Parathion	1	26	1	24	0	25	5	19
Malathion	26	21	33	40	29	29	23	31
Diazinon	17	40	1	22	0	17	25	9
Co-ral	2	41	2	16	0	21	25	13

However, evidence that cholinesterase recovery does in fact occur is provided by studies such as that of O'Brien (1956) on whole populations, i.e., living plus dead. Unhappily, the protective technique had not then been thought of. One can also utilize the present data for 320 and 1,280 minutes to find the average inhibition of living and dead flies, by summing the weighted average inhibitions at any one time. The weighting factors are the fractional survival and mortality, respectively. For cholinesterase one then finds recovery for Co-Ral (from 29% to 40% activity) and malathion (from 48% to 50%) but not for parathion (47% to 27%) or Diazinon®(*O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate) (44% to 36%). For phenyl acetate hydrolysis, recovery always occurred: for parathion, 22% to 26%; for malathion, 37% to 49%; for Diazinon, 17% to 29%; and for Co-ral, 9% to 11%.

Discussion.—If these results are added to those of van Asperen (1960) and Plapp & Bigley (1961), it seems safe to say that it is a general phenomenon, true of both head and whole-body enzymes that, in the early stages of house fly poisoning by organophosphates, the cholinesterase of survivors is inhibited less than any other esterase so far studied; and that cholinesterase inhibition by an LD_{50} is never so severe as the findings of Mengle & Casida (1958) had indicated. The latter authors used an unprotective technique, and found an average maximal cholinesterase inhibition in survivors of 84% with 16 organophosphates, used at the LD_{50}. In some cases the disparity is more marked: peak inhibitions for an LD_{50} of parathion are 96% (Mengle & Casida 1958) as contrasted with 50% in the present study and 46% (Plapp & Bigley 1961).

The most interesting finding is that in *dead* flies cholinesterase is severely inhibited (74% to 99% at 320 minutes) in every case, and that at 320 minutes its inhibition is in many cases equal to or greater than the inhibition of hydrolysis of the other esters. This observation radically changes the picture of the relative susceptibilities of cholinesterase and other esterases. It is perhaps surprising to think how many previous comparative studies have been directed to enzymes in surviving insects, when one really wishes to know what happened to the dead ones. The present results, however, are unexpected in view of the finding by van Asperen (1960) that those house flies *knocked down*, and thus not necessarily killed, by several organophosphates, have their cholinesterase inhibited far less than their "aliesterase." It may be that the factors responsible for knockdown are different from those responsible for death, as was previously suggested by Mengle & O'Brien (1960) to explain the finding that pyridine 2-aldoxime methiodide delayed paralytic symptoms after treatment with parathion.

Table 2.—Comparison of esterase levels in live and dead flies.

SUBSTRATE: TIME AFTER TREATMENT (MIN.):	RATIO: LEVEL IN LIVE/LEVEL IN DEAD					
	Acetylcholine		Phenyl Acetate		Tributyrin	
	320	1280	320	1280	320	1280
Parathion	67.0	2.3	26.0	1.0	6.4	0.8
Malathion	2.1	2.9	1.2	1.3	1.5	1.3
Diazinon	3.9	1.8	32.0	1.7	1.0	3.3
Co-ral	21.0	1.5	6.5	0.6	0.6	1.2

malathion and diisopropyl phosphorofluoridate, but had no effect upon the LD_{50} or upon inhibition of cholinesterase.

Another analysis of the data consists in comparing the esterases in living and dead flies, as in table 2 (the data for methyl butyrate are not included, because of the scatter of results of figure 1). For cholinesterase, survivors always have far more enzyme than the dead (maximum ratio 67, minimum 1.5). For phenyl acetate hydrolysis this is usually but not always true (maximum ratio 32, minimum 0.6) and for tributyrin hydrolysis it is even less true (maximum ratio 6.4, minimum 0.6). If one compares the ratios for any given insecticide at any one

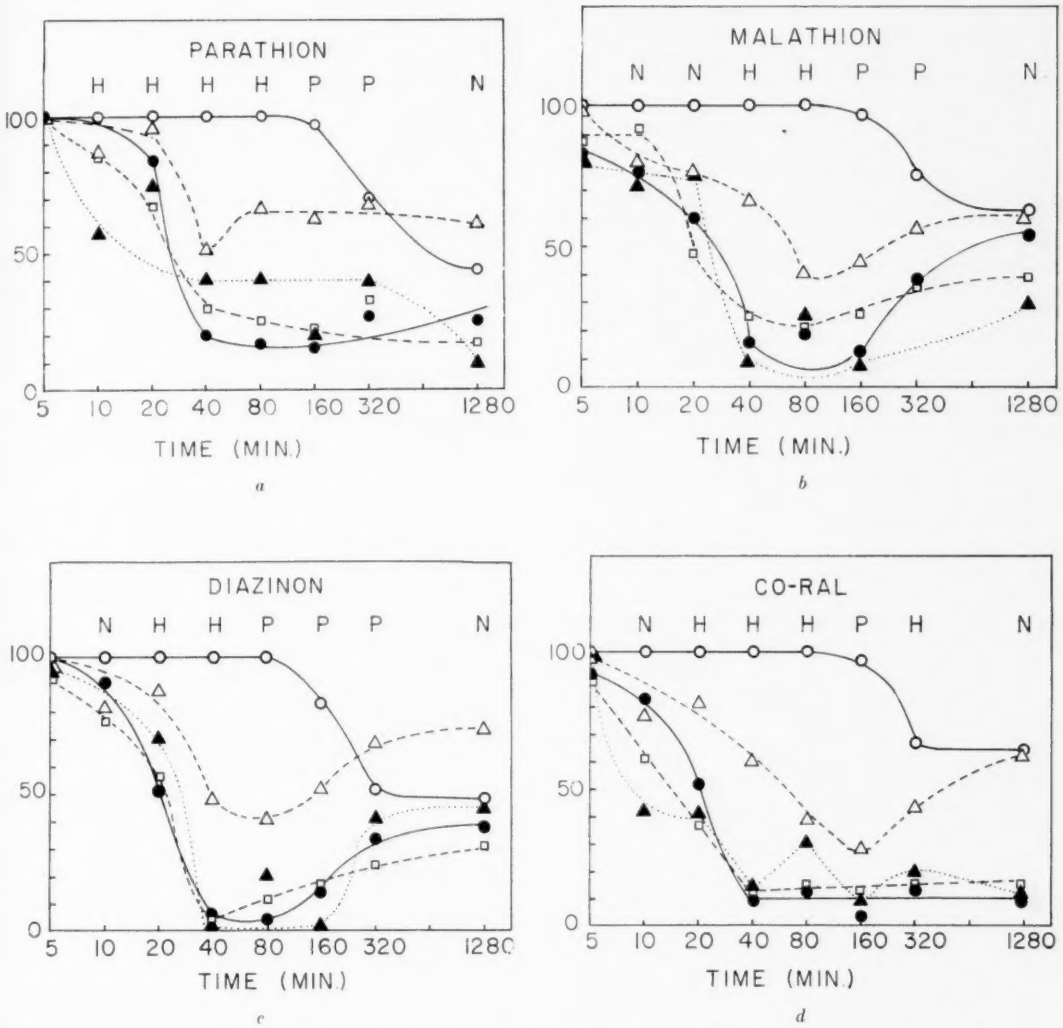

FIG. 1.—Symptoms, survival and esterase levels of survivors from an LD_{50} of parathion (1.0 mg./kg.), malathion (12.5 mg./kg.), Diazinon (1.5 mg./kg.) and Co-ral (3.0 mg./kg.). Symptoms: N=normal, H=hyperactive, P=prostrate. These refer to the survivors at any given time. Per cent survival: ○———○. Esterase level in survivors, as per cent of normal: Phenyl acetate ●———●. Acetylcholine △- - - -△. Methyl butyrate ▲ . . . . . ▲. Tributyrin □— — —□.

time, then in 14 cases out of 16 the ratio for cholinesterase is greater than that for phenyl acetate or tributyrin. The two exceptions occur with Diazinon: in one case for phenyl acetate at 320 minutes, in the other for tributyrin at 1,280 minutes.

It is concluded that the death of flies from organophosphate poisoning is associated with and is probably due to, inhibition of cholinesterase rather than other esterases. Even with protective technique, severe inhibition of cholinesterase is produced in victims. The fact that the survivors are those which escape such severe cholinesterase inhibition, perhaps because of superior ability to degrade the poison, is supporting evidence for the importance of cholinesterase.

These findings are in harmony with those of Stegwee (1960), who showed that "aliesterase" can be virtually eliminated in house flies by a dose of crude triorthocresyl phosphate which has no ill effects upon the insects. The conclusion is in agreement with that of Plapp & Bigley (1961) but the nature of the evidence is quite different.

The remarkable recent finding that decapitation has little effect upon the LD_{50} for house flies of eight anticholinesterases (Mengle & Casida 1960) makes it improbable that inhibition of the cholinesterase of the head is important in poisoning. Consequently, the results of those studies in which *in vivo* observations are based purely upon inhibition of head cholinesterase (Mengle & Casida 1958, 1960, Mengle & O'Brien 1960, Plapp & Bigley 1961) should not be used in correlating symptoms with extent of cholinesterase inhibition. Since 67% of house fly cholinesterase is in the head (Stegwee 1960) studies on whole homogenates, as in the present study, may also be weighted by inappropriate evidence. Future work should therefore concern itself with inhibition of particular tissues other than head (the thoracic ganglion being a promising source) along the lines already begun by Stegwee (1960).

If it could be shown with a variety of organophosphates that death always occurs when the cholinesterase of some particular tissue is reduced below a certain critical level (and perhaps for some critical period of time), then we could accept as proven the hypothesis that organophosphates kill insects by inhibiting their cholinesterase.

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The Influence of Oil Viscosity and Timing of Treatment on Semidormant Control of European Fruit Lecanium[1]

E. H. Smith, *New York State Agricultural Experiment Station, Geneva,* and J. H. H. Phillips, *Canada Department of Agriculture, Vineland Station, Ontario, Canada*

Abstract

Paraffinic oils ranging in viscosity from 30 to 220 seconds (SUS at 100 ° F.) were employed in field tests in 1957–60 for control of the European fruit lecanium, *Lecanium corni* Bouché. The size of scales at the time of treatment was found to influence control efficiency, the scales being less susceptible as development advanced. During the interval from dormant to delayed dormant stage of bud development the weight of scales increased 8-fold and 4-fold, respectively, on apple and plum and this increase coincided with a decrease in control by oil sprays applied at these stages. Prior to commencement of growth oils well below 70 second viscosity proved effective. In one season data were obtained suggesting that control of approximately 95% of the overwintering scales was ineffective in preventing reinfestation and smutting of fruit at harvest.

The European fruit lecanium, *Lecanium corni* Bouché, has been controlled almost exclusively by semidormant oil sprays except during the past decade when oils were replaced by organophosphate sprays directed against newly hatched nymphs. More recently since the widespread occurrence of insect resistance to synthetic organic insecticides there has been renewed interest in the use of petroleum oils to which resistance has not occurred.

Despite their long period of usage conclusive data is lacking on precisely how effective petroleum oils are against this pest. Several factors contribute to this situation. Results have generally been based on the effect of treatments on the overwintering scales without regard to reinfestation before harvest by nymphs arising from the survivors. The species is subject to wide fluctuation in population levels due to natural factors. Consequently, a treatment may be credited with levels of control which actually were caused by natural factors. In addition, certain spray materials used after the semidormant treatment often contribute to control, thus obscuring the true effectiveness of the oil treatment.

The object of the present study was to reevaluate the effectiveness of oil sprays employing as a standard the light fractions of paraffinic oils of the type recommended in New York State (Chapman 1959). These oils have a viscosity of 70 seconds and it is hoped that even further reductions in viscosity can be made without sacrifice in effectiveness thus extending the usefulness of petroleum oils through reduced phytotoxicity. The present report covers field evaluations of petroleum oils over the period 1957–60. The influence of timing on effectiveness has been stressed during the past 2 years.

Materials and Methods.—Oils were tank-mixed using blood albumin as an emulsifier at the rate of one-half pound of the 25% product per 100 gallons. Sprays were applied with a hand gun operated from a hydraulic type orchard sprayer at 400 p.s.i. Trees were sprayed to the point of run-off. Oil deposit was determined by Dr. A. W. Avens of the New York State Agriculture Experiment Station, using the method of Pearce et al (1941). Samples of twigs for oil deposit determinations were collected as soon as practical after the spray dried.

Results were generally based on the number of live scales present several weeks after treatment although results by several methods are reported for comparative purposes. By postponing counts until several weeks after treatment the survivors could be readily distinguished with the unaided eye.

The experimental oils were paraffinic in nature and represented a viscosity (SUS at 100° F.) range of 30 to 220 seconds. Specifications for the oils are reported in table 1.

1957 Tests.—The object of these tests was to evaluate the effectiveness of four oil fractions ranging in viscosity from 59 to 106 seconds. The test orchard was a 20-year-old Fellenburg prune planting at Sodus, N.Y. The oils were employed at 1% and 2% concentrations and plots consisted of four trees each (2×2) replicated four times. Sprays were applied on April 23 when buds were in the green tip stage.

In addition to the usual counts made several weeks after treatment, counts were taken also at completion of hatch of nymphs arising from the survivors of the semidormant treatment. It was originally planned to make periodic counts throughout the season so that results based on reduction in overwintering scales could be interpreted in terms of population levels and smutting of the fruit at harvest. Such data would provide a basis for establishing the degree of semidormant control required to insure freedom from smutting at harvest. This plan had to be modified following the inadvertent treatment of the plots in mid-August by the grower with heavy concentrations of malathion and DDD for control of red-banded leaf roller (*Agryrotaenia velutinana* (Walker)). This treatment resulted in virtually complete kill of scales. When this complication was discovered in late August the plots were immediately rated on the still visible smutting which occurred prior to the accidental spraying.

In addition to control data, a report is included on experiments in sampling and counting in an effort to overcome some experimental difficulties posed by this species.

Counts of Overwintering Scales Present at Bloom.—Samples of branches were collected on May 6 when trees were in full bloom. In sampling, four branches were collected from each tree, one from each cardinal direction. The previous 2 years' growth was discarded and approximately 18 inches of older wood used for counts.

Results were based on counts of live and dead scales after allowing for natural mortality. In addition, results were also determined solely on reduction in live scales over the untreated. The latter method which can be

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Table 1.—Properties of petroleum oil fractions employed in control studies of the European fruit lecanium. 1957–1960.

Name or Code No.	Supplier	Viscosity SUS at 100° F.	Gravity API	UR % USTM	Distillation 10%	50%	90%
Certrex 478	Socony Mobil	30	46.8	89	410	429	453
Certrex 1102	Socony Mobil	43	36.9	88	542	581	652
Socony—XTX8	Socony Mobil	44	38.2	90	522	576	616
Prorex #1	Socony Mobil	57	36.6	94	612	646	712
LS-0441	Standard Oil Co. of Indiana	67	35.9	95	636	669	708
Sun X-88798	Sun Oil Co.	69	33.9	95	644	668	709
Sunvis 7	Sun Oil Co.	70	33.5	96	644	668	696
Prorex #2	Socony Mobil	106	32.0	92	670	701	750
Volck Supreme	California Spray Chem. Co.	142	31.0	95	—	—	—
Imperial	Imperial Oil Co.	220	27.5	70	715	810	930

accomplished without the aid of a microscope requires less counting time, but is less reliable when erratic distribution of the pest occurs. These results are shown in table 2. Results by both methods of counting were in close agreement indicating that the shorter method based on number of scales present rather than per cent live may be employed with accuracy in heavy infestations.

At 1% concentration control efficiency varied from 60% to 82% with Prorex #1 and Prorex #2 being most effective. All oils were significantly more effective at 2% than at 1% but only Prorex #2 was significantly different from the other oils at 2%. The relationship of viscosity to control is not clearly established by these data as the most effective oils at 1% represented the widest range in viscosity from 57 to 106 seconds. Although a small but significant difference in favor of the heavier oil at the 2% concentration was disclosed the oils did not differ greatly in effectiveness and it can be concluded that the breaking point in effectiveness of paraffinic oils of this type falls below 57 seconds viscosity.

Counts Following Completion of Hatch.—Nymph counts were made at the completion of hatch with the aid of a binocular microscope. In sampling leaves, the first full-sized leaf of the current season's growth was taken from a central terminal branch. These leaves are consistently the most heavily infested. Samples consisted of 100 leaves per plot.

Table 2.—Control of European fruit lecanium by semi-dormant oil sprays applied at early green tip stage of bud development on April 23, 1957, Sodus, N.Y.[a]

Oil	Viscosity	Concentration 1% Control Efficiency[b,d] (%)	1% Control[c] (%)	2% Control Efficiency[b,d] (%)	2% Control[c] (%)
Prorex #1	57	73 a	73	93 cd	91
Prorex #2	106	82 a	84	98 c	98
Sun Oil X-88798	69	60 b	58	92 d	92
LS-0441	67	69 ab	72	92 d	92

[a] Comparison of data based on two methods of calculating results.
[b] Average of 4 replicates based on count of live and dead scales. Eighty-eight per cent live in untreated on May 6.
[c] Average of 4 replicates based on reduction of live scales over untreated. Thirty-five scales per foot of branch in untreated on May 6.
[d] Means in a column followed by dissimilar letter are significantly different at 5% level.

To make critical comparisons between treatments as planned in this case requires thorough sampling of replicates. The time required to count all scales on heavily infested leaves restricts the number of leaves in each sample. Neither the brushing technique employed in mite counts nor the Berlese-funnel method employed for aphid counts was satisfactory in collecting the attached nymphs. This problem was overcome by counting the scales on a disk $\frac{7}{16}$ inch in diameter cut from each leaf with a cork punch pressed against a rubber stopper. Leaves could be stacked, punched and discs transferred to Syracuse watch glasses. The samples could then be stored in a refrigerator for later counting. The addition of moisture to the covered watch glasses kept the discs in a turgid condition. The $\frac{7}{16}$-inch disc just filled the visual field of the binocular at the magnification employed, so that error involved in overlapping fields was overcome. Discs were taken from the basal end of the leaf between the outer margin and the midrib. The midrib was avoided as scales along it were not readily visible. Conversion of disc counts to "scales per leaf" could not be made on the basis of relative area because the basal area of the leaf is most heavily infested. A conversion factor was derived by comparing the average scales per leaf by actual count with the average scales per disc. Accordingly, the disc count multiplied by 13.3 approximated the leaf count.

The effectiveness of treatments based on overwintering scales present at bloom and on nymphs present after completion of hatch are shown in table 3. The two methods of making nymphs counts are reported. Expressed as percentage, the relationships carry over quite consistently from counts of survivors present at bloom to posthatch counts of nymphs arising from those survivors. There is also good agreement between results based on leaf and disc counts. The striking feature is the high nymph populations even at the highest levels of control. It is apparent that the density of population of nymphs is directly related to the number of survivors on the individual tree with no suggestion of spread from heavily infested trees.

Mid-Season Counts.—All but one replication was treated at the completion of hatch to avoid excessive injury to the planting. Periodic counts were to have been made on this replication until harvest, but as previously reported this plan had to be modified because of the accidental spraying. The effectiveness of semidormant treatments at mid-August was based on amount of leaf smutting still visible a week after the accidental spraying.

Table 3.—Measures of effectiveness of semidormant sprays on European fruit lecanium based on counts of overwintering live and dead scales present at bloom (May 6), and nymphs present at completion of hatch (July 9), 1957.[a]

Oil	Viscosity	Concentration (%)	Counts at Bloom: Control[b] Efficiency (%)	Counts at Completion of Hatch: Nymphs per Leaf	Control (%): Whole Leaf Count	Control (%): Leaf Disc Count
Prorex #1	57	1	73	136	73	75
		2	93	54	89	91
Prorex #2	106	1	82	59	88	93
		2	98	18	96	95
Sun Oil (X-88798)	69	1	60	169	67	66
		2	92	36	93	90
LS 0441	67	1	69	102	80	78
		2	92	32	94	93
Untreated (No. live scales)			—	514	514	38

[a] Nymphs counts based on nymphs per leaf and nymphs on 7/16-inch discs cut from leaves.
[b] Based on 88% live scales in untreated.

Samples of 100 leaves collected at random from each plot were scored according to the degree of smutting. A value of 0, 2 and 4 was given to leaves classified clean, light and heavy. The degree of smutting could then be expressed as per cent reduction as compared with leaves from untreated trees. Results obtained in this manner are compared with earlier counts of scales per leaf at completion of hatch in table 4. Striking differences in degree of smutting occurred. Half of the leaves from the untreated plot were heavily smutted. Two of the treatments which gave approximately 60% reduction at completion of hatch showed no reduction in leaf smutting. Reductions of 69% or greater at completion of hatch were reflected in some reduction in smutting but in no case was smutting completely eliminated. It is likely that smutting would have grown progressively worse by harvest in early September had the scales not been killed in mid-August.

The striking feature of these data is that while semidormant oil treatments result in control of the order of 95% the survivors gave rise to large numbers of nymphs which reinfested at levels sufficient to cause smutting. It therefore becomes questionable whether the degree of effectiveness obtained by semidormant treatment provides practical control. It is recognized that the test orchard represented an unusually heavy infestation and that these results of a single season may be atypical. Observations extending over several years are needed to establish the relationship between semidormant control and effectiveness at harvest. While the effectiveness of the treatments remains in doubt it is apparent that the oils of the viscosity range represented are of essentially equal effectiveness.

1958 TESTS.—These tests were designed to compare the effectiveness of the two principal oils tested the previous year with one of lower viscosity. The last product selected was a specially prepared fraction having a viscosity of 44 seconds (Socony XT48). Treatments were made on May 5 when trees were in the delayed dormant stage. The test orchard was a 15-year-old Fellenburg prune planting at Williamson, New York. Plots 2 rows wide and 3 to 5 trees long were replicated 2 to 3 times. Results were based on reduction in live scales present at bloom. Sampling and counting methods were as described in the foregoing. The results of these counts are given in table 5. All treatments of equal concentration gave essentially the same degree of control regardless of viscosity. Prorex #1 and Prorex #2 gave significantly less control than in 1957. The only obvious factor that differed in the two seasons was timing of sprays, the sprays in 1957 having been applied at green tip as compared with delayed dormant in 1958. It was suspected that at the time of treatment the scales were larger in 1958 than in 1957 and that the larger scales were less susceptible to oil treatment.

1959 TESTS.—Plans were made to follow up the leads of the previous season and compare the effectiveness of a viscosity series applied at dormant, green tip and delayed dormant stages of bud development. This plan had to be modified to include only two timing treatments because of unseasonably warm weather which advanced bud development beyond the dormant stage before the first treatment could be made.

Table 4.—Semidormant control of European fruit lecanium based on counts of nymphs at completion of hatch and leaf smutting present in mid-August, 1957 (Replicate 1 only).

Oil	Viscosity	Concentration (%)	Completion of Hatch (July 9): Scales per Leaf	Completion of Hatch (July 9): Control (%)	Leaf Smutting in Mid-August: Leaf Smutting (%) Clean	Slight	Heavy	Sum[a]	Reduction (%)
Prorex #1	57	1	91	69	31	44	25	188	27
		2	64	79	51	32	17	132	49
Prorex #2	106	1	76	75	57	29	14	114	56
		2	35	88	67	22	11	88	66
Sun Oil (X-88798)	69	1	121	60	13	32	55	284	0
		2	13	96	50	41	9	118	54
LS 0441	67	1	128	57	9	27	64	310	0
		2	31	90	75	21	4	58	77
Untreated			298		21	29	50	258	—

[a] Number of leaves in each class multiplied by weighed score: Clean—0, slight—2, heavy—4.

Table 5.—Control of European fruit lecanium on prune by semidormant treatment with petroleum oils. Williamson, New York.

Oil[a]	Viscosity	Concentration (%)	Control (%)
Socony XTY8	44	1	33[b]
		2	78[b]
Prorex #1	57	1	41[b]
		2	83[b]
Prorex #2	106	1	39[c]
		2	77[c]
Untreated, live scales per ft. of branch			46[c]

[a] Sprays applied at delayed dormant stage on May 5, 1958.
[b] Average of 2 replicates.
[c] Average of 3 replicates

The experimental orchard was a 30-year-old Ben Davis apple planting located at Red Creek, New York. The infestation was variable over the orchard and three sections most consistently infested were selected for use in the timing series. Single plots consisted of 10 randomized trees and counts were based on live scales on 10 branches from each tree. Results were expressed as per cent reduction in scales per branch unit as compared with untreated. It would have been desirable considering the uneven distribution of the infestation to base results on counts of live and dead scales but this plan was thwarted by the dislodgement of the dead scales before counts could be completed, a complication which did not arise in the 1957 test.

Sprays were applied on April 21 when buds were in an early green tip stage and on April 30 at delayed dormant. Development during this interval was accelerated by unusually high temperatures which reached a maximum of 80° F. Samples of scales were collected at the time of each treatment and measured to establish average size. The results of these tests are given in table 6.

It will be noted in the Sunvis 7 series that results of early and late treatment are based on comparison with the same untreated plot while in the other series a separate untreated plot served as the basis for comparison of the early and late treatment. This complication together with the lack of replications renders these data unsuited for establishment of firm conclusion but they are deemed useful in indicating leads for further study.

In all cases control was more effective in the early treatment. Using 95% as an arbitrary level of effectiveness, all but the lightest oil (30 seconds viscosity) were effective in early treatment while at the later application none was effective. Within the range employed the heavier oils were more effective but the breaking point varied depending on the time of treatment. Actual oil deposit has repeatedly proven a more reliable dosage criterion than concentration of oil in the spray mixture (Chapman et al. 1941). Limited oil deposit data for 1% and 2% concentrations of Sunvis 7 establish the following.

	Mg. oil per 100 sq. cm.	
	April 21	*April 30*
Sunvis 7 1%	3.7	3.4
Sunvis 7 2%	7.1	10.5

Table 6.—Control of European fruit lecanium on apple by semidormant treatment with petroleum oils.[a] Red Creek, N. Y. 1959.

Oil	Viscosity	Concentration (%)	Per Cent Control—Timing: Green Tip	Per Cent Control—Timing: Delayed Dormant
Sunvis 7	70	1	45	27
		2	96	65
Untreated (scales per ft.)			2.9	2.9
Volck Supreme	142	2	99	91
Prorex #1	57	2	97	29
Certrex 1102	43	2	95	23
Certrex 578	30	2	40	0
Untreated (scales per ft.)			12.2	5.9
Size of scale in mm:				
length			1.20	1.61
width			0.66	0.92

[a] Single sprays were applied at green tip (April 21) and delayed dormant (April 30) to compare influence of timing on control.

The deposits are slightly lower than are generally expected likely owing to delay in collecting samples but the data appear to eliminate deposit as a variable accounting for difference in control by early and late treatment.

The most likely factor accounting for difference in control is size of scale at time of treatment. The true size differences are of course greater than is reflected in the linear measurements given (table 6). The greatest change is in volume which would be more accurately revealed by data on weight.

1960 TESTS.—These tests were undertaken to gain additional data on the relationship of timing of sprays and size of scale to effectiveness. Sprays were again applied at dormant and delayed dormant stages of bud development and the weight of scales was determined at intervals as the season advanced. Two to seven samples of 100 scales each were weighed at each collection to establish average weight.

Tests were established in an apple and plum orchard. The apple orchard was a 45-year-old planting of Northern Spy located at Simcoe, Ontario, Canada. Plots one row wide and five trees long were replicated four times. Counts were taken on live scales present after bloom on ten 18-inch branches from each tree. The plum orchard, which was located at Vineland, Ontario, was an 18-year-old planting of mixed Japanese and European varieties. Plots consisted of 4 trees each (2×2) and were replicated 4 times. Sampling and counting procedures were similar to those reported for apple. The schedule of sprays was as follows:

Host	*Date Sprayed*	*Stage*
Apple	April 13	Dormant
	May 5	Delayed Dormant
Plum	April 23	Dormant
	May 3	Delayed Dormant

The results of these tests are given in table 7. The infestation in the apple planting was heavy throughout. The early treatment gave striking control at the 1%

Table 7.—Control of European fruit lecanium[a] with semidormant oil sprays in relation to size of scale and timing of treatments. Ontario, Canada. 1960.

Oil	Viscosity	Concentration (%)	Apple				Plum			
			Control[b] (%)		Oil Deposit[c]		Control[b] (%)		Oil Deposit[c]	
			April 13	May 5	April 13	May 5	April 13	May 5	April 13	May 5
Sunvis 7	70	1	96 c	78 d	7.9	7.8	90 a	44 b	4.9	4.0
		2	99 c	96 c	13.2	16.2	88 a	70 b	12.8	—
Imperial	220	1	99 c	76 d	9.5	8.1	—	—	—	—
Weight of scales mg. per 100			2.6	22.0			5.0	17.0		

[a] Scales per foot in untreated: Apple—15.0; plum—2.7.
[b] Means in a column followed by dissimilar letter are significantly different at 5% level.
[c] Oil deposit expressed in mg. per 100 sq. cm.

concentration. At both concentrations, the late treatment was less effective than the early one. It is interesting to note that the high viscosity oil is about equal in effectiveness to the low viscosity oil at comparable deposits.

The infestation in the prune planting was light but uniformly distributed. An unexplained inconsistency occurred in the early treatment, 1% giving slightly higher control than 2%. As in the case of apple, the late treatment was less effective than the early one. The higher control on apple is possibly due to heavier oil deposit.

The relationship between weight of scales, timing of sprays and control is shown graphically in figure 1. The relationship between host bud development (Stanley Plum) and size of scale is shown in figure 2. Little or no growth is believed to have occurred prior to the application of the earliest spray. This assumption is based on weighings made in January 1961 while the scales were clearly still dormant. The average of five weighings gave values of 4.64 mg. per 100 scales from plum and 2.45 mg. from apple. These values are essentially equal to weights determined in April 1960. The 2-fold difference in weights of scales on different host plants is believed due to a varietal difference occurring within the *Lecanium corni* complex.

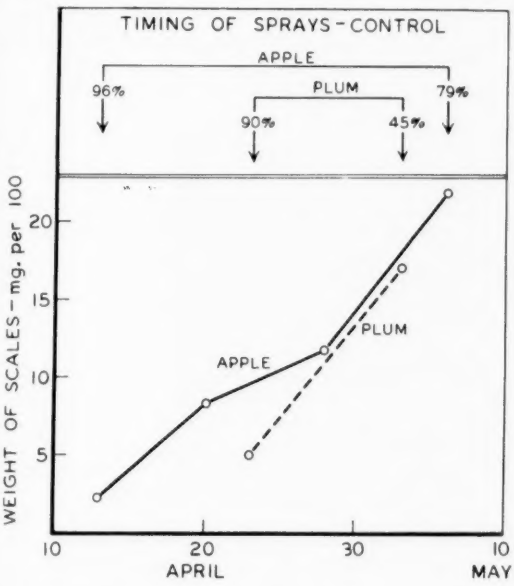

FIG. 1.—Showing control of European fruit lecanium with 1% oil spray in relation to weight of scale and timing of sprays.

In the interval between sprays the scales on apple increased in weight 8.4-fold while those on plum increased 3.5-fold. Precise comparisons between results on apple and plum are not readily made because of variability in oil deposit as well as weight of scales. Control data obtained from both host plants confirm the indications of the previous season that susceptibility to oil decreases as the season advances once growth of the scales commences.

Exploratory tests were conducted in the green house to determine rate of change in susceptibility. While these tests met with unforeseen experimental difficulties they provide some interesting leads for further study (table 8). The greatest change in susceptibility occurred between the treatments on April 28 and May 6. Lower concentrations of oil might have revealed a gradual change in susceptibility to the earlier treatments. The data at hand suggest that beyond a critical size susceptibility to a standard concentration of oil decreases rapidly.

Apparently scales on different host plants do not differ greatly in their response to oil treatment. Greater differences in control can be attributed to different rates of oil deposit than to any physiological influence of the host plant.

In pest species which are characterised by wide fluctuations in natural mortality it might be suspected that high natural mortality would predispose the survivors to

Table 8.—The susceptibility of European fruit lecanium to oil sprays. Infested apple branches treated and held in greenhouse at approximately 80° F. Vineland, Ontario, Canada, 1960.

Oil	Viscosity	Concentration	Per Cent Control[a] on Date Shown			
			April 13	April 20	April 28	May 6
Sunvis 7	70	1	94 a	92 a	100 a	70 b
Weight of scales[b] (mg./100)			2.6	9.4	11.1	22.0
Stage of bud development			Dormant	Dormant	Dormant	Delayed Dormant

[a] Means followed by dissimilar letter are significantly different at 5% level.
[b] Average of 10 replicates.

FIG. 2.—Showing size of European fruit lecanium in relation to bud development of Stanley Prune.

greater susceptibility to treatment. The evidence obtained in these studies on natural mortality and its influence on susceptibility is not conclusive but is deserving of consideration. In the 1960 studies natural mortality was of the same order on both host plants as evidenced by the following:

	Scales per Foot of Branch	
Date	*Apple*	*Plum*
April 12	107.6	50.8
June 16	21.4	3.6
Mortality (%)	82	92

The greatest mortality appears to be a direct result of winter conditions as relatively little mortality occurred after active growth began. On apple the total natural mortality was 69% on April 12 and reached 77% by May 13. The control obtained in 1960 (table 7), a season of high natural mortality, is not strikingly different from control obtained in 1957 (table 2) when natural mortality was of the order of only 12%.

DISCUSSION.—In attempting to account for differences in control as cited in the foregoing, three areas of variability are considered:

1. Oil deposit at time of treatment.
2. Weather conditions following treatment.
3. Variable susceptibility of the pest.

The oil deposit data eliminate this factor as the variable accounting for the results cited. It should be stressed that critical comparisons of the effectiveness of oils requires data on oil deposit rather than concentration of oil in the spray mixture. Wetting agent, weather conditions, spray equipment and the human element all influence actual deposit after the concentration is established.

Weather conditions following treatment may conceivably influence control through its effect upon volatility of the oil film or through an effect on the pest. In 1959 and 1960 the weather following each spray was not markedly different and it seems unlikely that normal extremes in temperature would influence volatility rates sufficiently to greatly alter effectiveness. While the rate of metabolism of the insect is regulated within limits by temperature no differences could be cited which appeared to explain the differential control.

Variable susceptibility appears to be the factor accounting for decreased effectiveness of oil treatment as the season advances. The European fruit lecanium is characterized by rapid growth in the spring coincident with the development of the host plant. In the interval from early bud development to bloom the scale progresses from the small overwintering form to maturity. In the course of about 10 days during this period weight of scales may increase 5-fold or more.

In addition to the increase in size it is likely that even more striking changes occur in metabolic activity as the insect goes from dormancy to an actively growing stage. These metabolic changes would be reflected in respiratory rate which presumably is directly related to mode of action of oil in some manner.

It is generally accepted in toxicological work that larger animals require greater dosages to produce equal mortality and this principle appears to account for decreasing susceptibility as the season advances. In view of this consideration the original objective of this study, to establish the relationship of viscosity to effectiveness, cannot be accomplished without regard to the size of scale at time of treatment. If sprays are applied in the dormant stage, oils well below the 70-second viscosity now specified can be used at standard concentration without sacrifice in effectiveness although it should be pointed out that the usual levels of effectiveness of the order of 95% control in semidormant treatments may not be high enough. The situation regarding this pest in which susceptibility declines as the season advances obviates one advantage envisioned for the lighter oils. Thus the chief impetus in development of lighter oils was the possibility of using them later in the season as the trees approached bloom. In the case of eggs of the European red mite (*Panonychus ulmi* (Koch)), susceptibility to oil increases as the eggs approach hatch in the delayed dormant stage (Chapman & Pearce 1949). The diverging susceptibility of the two species is of practical interest as

a single spray is often applied for control of both. Obviously optimum control of both species cannot be had with a single spray and some compromise in control efficiency may be required.

It is apparent from these studies that the fullest employment of oils requires better understanding of the biology of the pest and the manner in which oils effect kill.

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Biological Studies of a New Greenbug Biotype[1]

E. A. WOOD, JR., *Entomology Research Division, Agric. Res. Serv., U.S.D.A., and Oklahoma Agricultural Experiment Station*

ABSTRACT

A greenbug (*Toxoptera graminum* (Rondani)) biotype has developed in greenhouse cultures at Stillwater, Okla. It is capable of destroying the resistant wheat lines Dickinson Sel 28-A., and C.I. 9058. When cultured on Dickinson Sel 28-A for eight generations, greenbugs collected from field populations decreased in size, fecundity, and longevity, whereas those of the new biotype remained normal.

In the fall of 1958, resistant wheat hybrids of Dickinson Sel. 28-A X Ponca and other resistant wheat lines were severely injured by the greenbug (*Toxoptera graminum* (Rondani)). Investigations indicated that the greenbug had either evolved a new biotype or was instrumental in transmitting a disease. To determine which factor was responsible, a series of experiments was initiated in 1959 in the laboratory in Stillwater, Okla.

The two strains of greenbug were cultured separately on Ward barley. One strain was collected from a wheat field near Fairview, Okla., and the other from cultures of greenbugs maintained in the greenhouse for the conducting of tests. In a preliminary experiment, two flats containing the two resistant wheats, Sel. 28-A and C.I. 9058, one susceptible wheat variety Ponca, and resistant Omugi barley were infested separately with each greenbug strain and enclosed in muslin-covered frames to prevent migration. Within 1 week all wheat varieties infested with the greenhouse strain were injured beyond recovery, but Omugi barley was not damaged. Dickinson Sel. 28-A and C.I. 9058 wheats and Omugi barley infested with the field strain sustained little or no damage, but the susceptible variety Ponca was severely injured (fig. 1).

DISEASE OR BIOTYPE.—Several tests were initiated to determine whether a disease or a biotype was responsible for the loss of resistance. In one test five adults of the greenhouse strain were cultured on Sel. 28-A for different lengths of time. They were then removed and replaced with five specimens of the field strain. At intervals, ranging from 3 to 10 days, these insects and also specimens of the greenhouse strain were transferred to Sel. 28-

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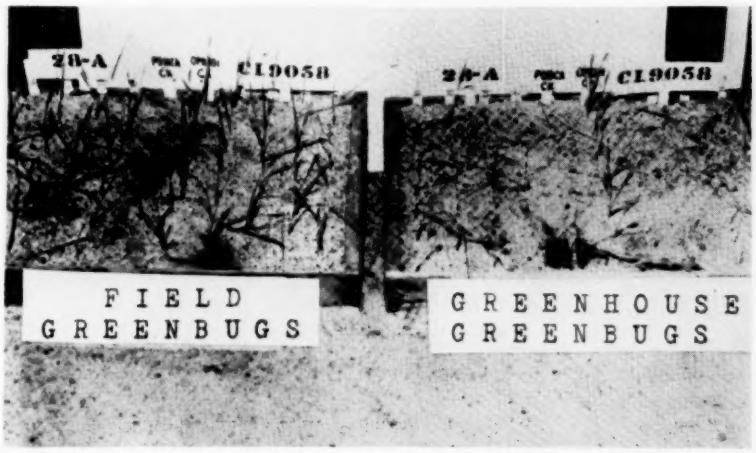

FIG. 1.—Resistant wheat and barley lines 7 days after infestation with the field and greenhouse strains of greenbugs.

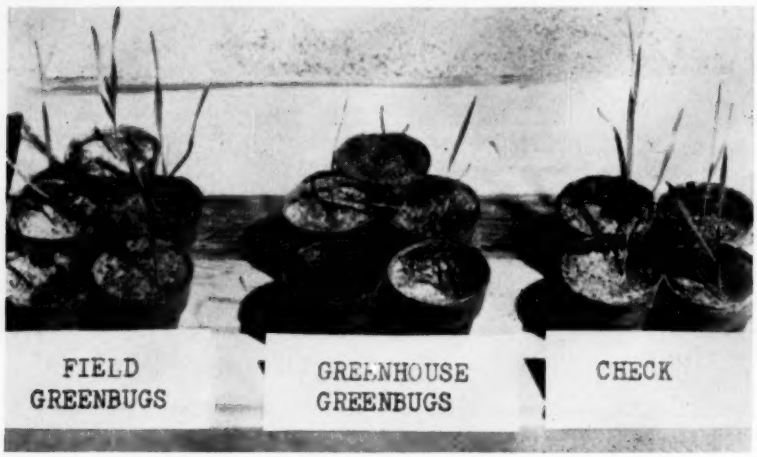

FIG. 2.—A comparison of damage to resistant Dickinson Sel. 28-A wheat 8 days after infestation with the field and greenhouse strains.

A seedlings for observation. The height of all plants was recorded prior to and again at the expiration of each experiment. Injury ratings were made periodically. These ratings were based on the amount of leaf injury observed on each plant. They ranged from 0, if no leaves were injured, to 5, if all leaves were destroyed. Sel. 28-A seedlings infested with the greenhouse strain became stunted after several days, and usually died within 10 days if the aphids were not removed. Plants from which the aphids were removed made a rapid recovery. No injury was noted on the seedlings infested with the field greenbugs that had previously fed on plants damaged by the greenhouse strain. The growth and vigor of these plants was comparable to the uninfested check plants (fig. 2). In an additional test, adults of the greenhouse strain were confined in a vial for several days without food. The nymphs produced from these specimens were used to determine whether a toxic substance was obtained from the plant while feeding with adults on the same plant, or if the ability to injure resistant plants was inherited. These previously unfed nymphs were placed on Ward barley and reared to maturity, then transferred to Sel. 28-A seedlings. They proved to be as toxic to resistant plants as other cultures of the greenhouse strain.

Another test involved the caging of both strains on two separate leaves of the same Sel. 28-A plant for 7 days and then transferring them to resistant seedlings. Within 18 days the seedlings infested with the greenhouse strain were given an injury rating of 5 and had gained an average of 33 mm. in height. Seedlings infested with the field strain were given a rating of 2 and had attained an average gain of 137 mm. in height. The uninfested check plants had gained an average of 160 mm. These tests seem to indicate that a new biotype and not a disease was responsible for the amount of damage caused on Sel. 28-A.

When eight generations of both strains of greenbugs were cultured on Sel. 28-A, the field strain, especially the winged individuals, had decreased considerably in size. Twenty specimens of each strain, including winged and wingless greenbugs, were taken at random, chloroformed, measured, and weighed. The field strain was much smaller in all dimensions and weighed half as much as the greenhouse strain. When both strains were cultured on Ward barley, their weights were comparable (table 1; fig. 3).

FECUNDITY AND LONGEVITY.—To obtain comparative fecundity data for the two strains, two tests were con-

Table 1.—Size and weight of greenhouse and field greenbugs cultured on barley and wheat for eight generations.[a]

GREENBUG STRAIN	AVG. BODY LENGTH (MM.)		AVG. BODY WIDTH (MM.)		AVG. WEIGHT (MG.)		AVERAGE WING-SPREAD (MM.)
	Winged	Wingless	Winged	Wingless	Winged	Wingless	
			Sel. 28-A wheat				
Greenhouse	1.56	1.30	0.50	0.68	0.24	0.12	2.00
Field	1.27	1.18	0.34	0.65	0.21	0.14	1.95
			Ward barley				
Greenhouse	1.26	1.24	0.50	0.40	0.17	0.16	1.95
Field	1.35	1.22	0.67	0.60	0.24	0.22	1.80

[a] Average of 10 greenbugs.

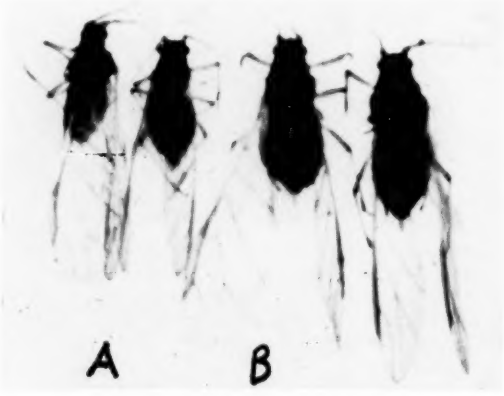

FIG. 3.—Comparative size of the field strain (A) and the greenhouse strain (B) after feeding on resistant Dickinson Sel. 28-A wheat for eight generations.

Table 2.—Fecundity and longevity of two strains of greenbug cultured on wheat and barley for 6 months.[a]

Greenbug Strain	Culture Host	Test Variety	Life Span (days)	Number of Progeny
Field	Sel. 28-A	Sel. 28-A	42	41
		Ward	55	61
	Ward	Sel. 28-A	47	28
		Ward	52	72
Greenhouse	Sel. 28-A	Sel. 28-A	46	67
		Ward	48	67
	Ward	Sel. 28-A	51	66
		Ward	48	58

[a] Average of 5 replications and 3 tests.

ducted. In the first, one young adult of each strain that had been cultured on Sel. 28-A wheat for eight generations was caged on Ward barley and Sel. 28-A wheat. After 5 days the cages were removed and the nymphs counted. The field strain produced an average of 8.2 nymphs on each host, whereas the greenhouse strain averaged 17.5 on barley and 14.5 on wheat. The reduction in fecundity indicated plant antibiosis to the field strain.

A second test to study longevity as well as fecundity involved aphids of both strains that had been reared for 6 months on Sel. 28-A wheat and Ward barley.

To obtain specimens of uniform age, several adults of each strain were transferred to and allowed to reproduce on caged seedlings of Ward barley and Sel. 28-A wheat grown separately in small flower pots. After 24 hours all adults were removed. The progeny thus obtained were from 1 to 24 hours old. Shortly before the last-instar stage was attained, all aphids except one were removed from each cage. These single aphids were observed daily to determine when reproduction began. After reproduction occurred the nymphs produced were counted and removed periodically. This process was continued until death of the original parent aphids terminated the experiment.

As noted in table 2, a very significant reduction in reproduction of the field strain occurred whenever Sel. 28-A was used as the test plant. The greatest reduction occurred when the field strain was cultured on Ward barley and transferred to Sel. 28-A. Although apparently normal for several days, reproduction sharply declined within 2 weeks. This same field strain, cultured entirely on Sel. 28-A, reproduced at a very low but constant rate throughout the experiment. When cultured on Sel. 28-A wheat and transferred to Ward barley this strain began to reproduce at a normal rate. The greenhouse strain reproduced normally on both host plants.

Longevity of the field strain decreased significantly when cultured on and transferred to Sel. 28-A. When transferred to Ward barley longevity was increased. The greenhouse strain was affected very little by either host plant; however, longevity was slightly reduced when Sel. 28-A was used as both the culture and the test host variety.

Specimens of both strains submitted for identification did not have any morphological differences and were classed as typical greenbugs. The only reliable method for distinguishing between the two strains is the reaction of resistant wheat lines to the feeding of each strain.

Systemic Insecticides for Six-Spotted Leafhopper Control and Reduction of Incidence of Lettuce Yellows[1]

L. S. THOMPSON and W. A. RAWLINS, *Cornell University, Ithaca, New York*

ABSTRACT

Three systemic insecticides, phorate, dimethoate and Di-Syston® (*O-O*-diethyl *S*-2 (ethylthio)ethyl phosphorodithioate), were investigated for six-spotted leafhopper control and prevention of the lettuce-yellow virus disease in Oswego County, New York, during 1958–1960. Results showed that significant reduction in the yellows incidence was possible with all three systemics when applied as soil treatments at a rate of 1.0 pound actual per acre at the time of planting. Treatments with granules or drenches were more effective than seed treatments and equally or more effective than malathion spray treatments. Time-mortality studies at the laboratory indicated that phorate was considerably more effective in killing leafhoppers in a 24-hour period than either Di-Syston or dimethoate. The systemic materials were effective 4 or 5 weeks following soil applications, but appeared to lose much of their effectiveness 6 weeks after application.

During the past 30 years several measures for the control of the six-spotted leafhopper, *Macrosteles fascifrons* (Stål), a vector of the lettuce-yellows virus, *Chlorogenus callistephi* Holmes, have been developed to reduce incidence of the virus disease. Of these methods the use of insecticides has been the most widely practiced in New York State lettuce plantings. The recommended chemical control schedules have reduced leafhopper population but have not prevented entirely inoculation of lettuce plants by viruliferous insects. The insecticides reduced the incidence of lettuce yellows by killing leafhoppers before they became viruliferous and by shortening the infection potential of immigrating viruliferous leafhoppers. However the toxic residues on the lettuce plants were only slowly effective and some inoculation took place before the vectors were incapacitated by the poison.

In order to keep the incidence of lettuce yellows at a minimum, insecticide applications must begin when plants are very young and must be continued at regular 5- to 7-day intervals throughout the first 6 weeks of the growing season. During some years the leafhoppers are so numerous that insecticide applications are necessary every 2 to 3 days, and on occasion, treatments must be repeated daily to reduce reinfestation by populations migrating from adjoining cultivated fields or pastures.

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Consequently control programs are relatively expensive, involving considerable costs for insecticides and for labor. Systemic insecticides applied as soil treatments, if equally as effective as foliar applications, would be less expensive than a schedule of sprays or dusts. Studies were made during 1958 through 1960 in lettuce fields to determine the efficacy of systemic insecticides. The principal producing area where the tests were conducted is the muckland of Oswego County, New York.

MATERIALS AND METHODS.—Only one systemic insecticide, phorate, was tested in 1958, comparing an 8% granular formulation and a 47.5% emulsifiable concentrate (4 pounds active ingredient per gallon). In addition to phorate two other systemics, dimethoate and Di-Syston® (*O,O*-diethyl *S*-2-(ethylthio)ethyl phosphorodithioate) were compared in 1959 and 1960, either as emulsifiable concentrates or granules. In 1960 standard and "coated" phorate and Di-Syston granules were tested, the latter type of formulation bearing a coating of charcoal powder impregnated with the systemic. For comparison, malathion emulsifiable concentrate (5 pounds active ingredient per gallon) was employed in frequent foliar sprays.

The insecticides were applied in two different ways: as seed treatment, and in-the-row applications of drenches or granules. The seeds (Cornell 456) were treated by pouring a 4% methocel solution over the dry seeds in a flask and shaking vigorously to distribute the sticker uniformly over the seed coats. Technical phorate was added at the rate of 1 pound per 10 pounds of seed. Again the seeds were shaken. Captan fungicide (1 pound to 10 pounds of seed) and activated charcoal (1 pound) were added as a mixture to the moistened seeds. A final shaking distributed the fungicide and charcoal over the seeds. The latter was included in the coating to reduce phytotoxicity of the phorate.

Soil treatments were applied using a hand-propelled garden planter redesigned to operate in tandem two hoppers, one for the seeds and one for granules. In operation the seeds and granules were metered from the hopper and conducted by separate chutes to the opened furrow.

In addition, the planter carried a small 1½-gallon tank for the drench solutions. Emulsifiable concentrates of the toxicants were diluted in water and applied in the row at the rate of 100 gallons of dilution per acre. Foliar applications were made with a low-pressure sprayer mounted on a garden tractor.

Two methods were used for estimating the performance of the various insecticides: (1) incidence of lettuce yellows at harvest time was the criterion employed for evaluating the efficacy of the insecticides. A satisfactory method for collecting leafhopper in lettuce plantings to ascertain populations has not been devised; hence the indirect approach of counting diseased plants was used as an alternative recourse. (2) to supplement this evaluation and to determine the length of time that the treated crop remained toxic to six-spotted leafhoppers, lettuce plants were moved from the field plots to the insectary where they were caged and infested with leafhoppers. Observations of mortalities were than made at 24- to 72-hour intervals.

Plants were transplanted from the field 4 to 6 weeks after comeup by placing a metal cylinder over a plant chosen at random and plunging the cylinder into the muck soil. When the cylinder was lifted the plant, in a core of soil, remained in the cylinder. The metal containers were made from discarded quart-size motor-oil cans, ends removed and thoroughly cleaned. With this procedure transplant "shock" was reduced to a minimum and the plants appeared to grow normally.

Six-spotted leafhoppers reared on barley in the insectary were confined to the plants in dacron marquisette-(25×32 threads per square inch) covered cages. Test plants were held in outdoor insectaries sheltered from wind and rain.

Table 1.—Methods of application, rates of phorate and lettuce-yellows incidence. Experiment 1, 1958.

METHOD OF APPLICATION	TOXICANT PER ACRE (LB.)	MEAN PERCENTAGE LETTUCE YELLOWS[a]
Drench	1	18
Granules	2	18
Drench	4	21
Drench	2	22
Seed	1 lb./10 lb. seed[b]	33
Untreated	—	38

[a] Means flanked by the same line are not significantly different at the 5% level according to Tukey's h.s.d. test.
[b] Orthocide (75 Captan) and activated charcoal included in seed treatment.

FIELD EXPERIMENTS.—*Experiment 1, 1958.*—Lettuce plants were arranged in a randomized complete block design with four replicates, each plot being 25 feet long and four rows wide. The results in table 1 show that phorate was effective in reducing the incidence of lettuce yellows when applied as granules in the drill at 2.0 pounds actual per acre or as a drench at 1.0 to 4.0 pounds actual per acre. Both granular and drench-type treatments were better than the seed treatment.

Experiment 2, 1959.—Another small-plot field experiment was conducted in 1959 to determine the effectiveness of three systemic insecticides in the prevention of lettuce yellows. Plot arrangements and plot sizes were the same as in the 1958 experiment. The three systemic insecticides, at all rates tested, were of significance in reducing the incidence of lettuce yellows (table 2). Slight delay in germination and a decrease in the number of seeds germinating were evident in most plots receiving treatments of systemic insecticides.

Experiments 3 and 4, 1960.—In order to obtain additional information on the effectiveness of the three systemic materials and to compare them with one of the conventional insecticides, malathion, two more field experiments were conducted in 1960. Regular Di-Syston and phorate granules were tested in the first experiment, and when samples of special coated granules of these insecticides were received, another experiment was conducted with these later acquisitions. Plots in both experiments were arranged in randomized complete block designs with four replicates, each plot being 40 feet long and eight rows wide in the first experiment and 50 feet long and five rows wide in the second. Two and one-half-foot fallow areas were left between each plot in the first experiment, and 5-foot fallow areas separated each replicate.

The incidence of lettuce yellows in all plots treated with systemics was significantly less than that in the

Table 2.—Methods of application, rates of insecticides and incidence of lettuce yellows. 1959 and 1960.

Insecticide	Method of Application	Toxicant per Acre (lb.)	Mean Percentage Lettuce Yellows[a]
	1959, Experiment 2		
Dimethoate	Granules	1	4.4
	Drench	2	5.1
Phorate	Granules	2	5.6
	Granules	1	7.7
	Granules	1.5	8.4
Di-Syston	Drench	1	8.7
	Granules	2	9.4
Phorate	Granules	0.5	10.6
Untreated	—	—	18.7
	1960, Experiment 3		
Di-Syston	Drench	2	2.7
Phorate	Granules	1	3.1
Di-Syston	Granules	1	4.0
Dimethoate	Granules	1	4.1
Malathion	Spray	1.25	4.3
Untreated	Untreated	—	8.4
	1960, Experiment 4		
Phorate	Granules (coated)	1	2.4
Dimethoate	Granules (regular)	1	2.7
Di-Syston	Granules (coated)	1	2.9
Malathion	Spray	1.25	4.9
Untreated	—	—	8.4

[a] Means flanked by the same line are not significantly different at the 5% level according to Tukey's h.s.d. test.

checks (table 2). In addition, Di-Syston drench at 2.0 pounds actual per acre in the first experiment, and phorate-coated granules at 1.0 pound actual per acre in the second experiment, were significantly better than malathion spray treatments. Poor germination was evident in all plots receiving systemic treatments, either as drenches or as granules in the drill.

TIME-MORTALITY TESTS TO SUPPLEMENT FIELD EXPERIMENTS.—In addition to records of incidence of lettuce yellows in the field plots as a criterion for evaluating insecticides, time-mortality studies were made at the laboratory using lettuce plants transplanted from the field plots in the manner described previously.

1958 Experiment.—Eight lettuce plants from each of the four phorate-treated plots and the untreated check plots were taken approximately 4 weeks after planting. Ten six-spotted leafhoppers were confined to each plant, mortality records being taken at the end of 24, 48 and 72 hours. Approximately 5 weeks after planting, leafhoppers were confined to another group of plants removed from the same field plots, mortality records being taken as with the first group of plants.

The plants from the phorate-treated plots were very toxic 4 weeks following application of the insecticide (table 3). Granules and drench treatments appeared to be much more effective than the seed treatment. Drill applications were almost as effective 5 weeks following treatment as they were after 4 weeks.

1959 Experiment.—Plants were removed from the 1959 field plots in the same manner as in 1958, approximately 4 weeks after application of insecticides and again after 6 weeks. Four weeks after application, phorate at dosages of 0.5 to 2.0 pounds actual per acre was considerably more effective in killing leafhoppers in a 24-hour period

Table 3.—Per cent mortality of leafhoppers confined to lettuce plants 4 and 5 weeks following application of phorate at planting time. Experiment 1, 1958.

Toxicant per Acre (lb.)	Method of Application	4 Weeks: Hours After Caging Insects 28	4 Weeks: 48	4 Weeks: 72	5 Weeks: 24	5 Weeks: 48	5 Weeks: 72
2	Drench	78	98	100	64	74	84
4	Drench	81	93	94	66	86	98
2	Granules	98	99	100	58	83	98
0.1	Seed treatment	18	40	56	—	—	—
Untreated	—	5	16	26	9	14	18

than was either Di-Syston or dimethoate (table 4). Two weeks later plants were much less toxic than at the previous sampling date. This may indicate a degradation of the chemicals in the soil or a decline in uptake by the plant.

1960 Experiments.—Plants were removed from the 1960 field plots approximately 5 weeks after application of insecticides in the first experiment and 4 weeks after application of insecticides in the second experiment. Phorate was somewhat more effective than either Di-Syston or dimethoate and appeared to kill leafhoppers more rapidly (table 4). Systemic treatments were more effective than the malathion spray, a spray having been applied 1 day prior to taking the plants from the field in the first experiment and 4 days in the second experiment.

DISCUSSION.—Chiykowski (1958) found that the long-residual systemic materials, demeton and American Cyanamid 12008, were very effective in the prevention of lettuce yellows in Wisconsin when applied as soil treatments on upland soil, but not on muck soils, where the systemics apparently became tied up in the soil and were not available to the plants. The results shown in

Table 4.—Per cent mortality of leafhoppers caged on plants 4 to 6 weeks following application of insecticides in-the-row at planting time.

Insecticide	Toxicant per Acre (lb.)	Method of Application	4 Weeks: Hours After Caging Insects 24	4 Weeks: 48	4 Weeks: 72	5 Weeks: 24	5 Weeks: 72	6 Weeks: 24
		1959 Experiment						
Phorate	0.5	Granules	84	—	100	—	—	13
	1	Granules	88	—	95	—	—	34
	1.5	Granules	93	—	100	—	—	33
	2	Granules	100	—	100	—	—	33
Di-Syston	2	Granules	38	—	100	—	—	30
	1	Drench	21	—	54	—	—	14
Dimethoate	1	Granules	36	—	79	—	—	8
	2	Drench	43	—	95	—	—	33
Untreated	—	—	10	—	18	—	—	13
		1960 Experiment 1						
Malathion	1.25	Spray[a]	—	—	—	25	44	—
Phorate	1	Granules	—	—	—	50	96	—
Dimethoate	1	Granules	—	—	—	33	70	—
Di-Syston	1	Granules	—	—	—	40	81	—
	2	Drench	—	—	—	30	63	—
Untreated	—	—	—	—	—	16	25	—
		1960 Experiment 2						
Malathion	1.25	Spray[b]	3	9	11	—	—	—
Phorate	1	Granules (coated)	62	100	100	—	—	—
Di-Syston	1	Granules	15	49	66	—	—	—
Dimethoate	1	Granules (coated)	13	23	33	—	—	—
Untreated	1	—	1	5	8	—	—	—

[a] Spray application 1 day prior to taking lettuce plants from field plots.
[b] Spray application 4 days prior to taking lettuce plants from field plots.

tables 1 to 4 indicate that this is not the case in New York, for significant reduction in the yellows incidence was possible with phorate, dimethoate and Di-Syston when applied at 1.0 pound toxicant per acre to muck soil at the time of planting lettuce. Increasing the dosage level above 1.0 pound had little effect either on the incidence of yellows or on the mortality of leafhoppers. Granules and drench-type treatments were more effective than seed treatments and equally or more effective than malathion spray treatments. However, both a delay and an increase in germination of lettuce seed was evident in most plots treated with systemic materials, especially when dry soil conditions existed.

Time-mortality studies indicated the relative speed at which leafhoppers were killed by the three systemic materials. Phorate was considerably more effective in killing leafhoppers in a 24-hour period than either dimethoate or Di-Syston. Insecticides were effective 4 or 5 weeks following soil applications, but appeared to lose much of their effectiveness 6 weeks after application. Coated granules did not appear to be any more effective than uncoated granules; however, the two formulations were not compared under similar conditions. If soil applications of systemic insecticides could be supplemented by 1 or 2 foliar applications with systemic insecticides or with one of the conventional sprays such as DDT, malathion, or parathion, it is believed that the incidence of lettuce yellows could be reduced even further, while keeping the cost of control at a minimum.

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Biochemistry of Malathion Resistance in *Culex tarsalis*[1]

F. MATSUMURA and A. W. A. BROWN,[2] *Department of Zoology, University of Western Ontario, London, Canada*

ABSTRACT

Larvae of the resistant strain of *Culex tarsalis* Coquillett, on exposure to malathion, come to contain one-third as much malaoxon as normal larvae. This is partly due to a higher phosphatase activity hydrolyzing malaoxon. But the major difference is in the higher carboxyesterase activity hydrolyzing malathion; this interstrain difference was demonstrated *in vivo* in larvae cleared of gut contents, and *in vitro* in homogenates and particularly the mitochondrial fraction. EPN, a carboxyesterase inhibitor, proved synergistic for malathion against the resistant strain. Increased carboxyesterase content was inseparable genetically from malathion resistance in hybridization experiments, which also indicated the malathion resistance to be due to a single partially dominant gene allele.

A population of *Culex tarsalis* Coquillett in Fresno county, California developed resistance to malathion applied as a larvicide from 1954 to 1956 (Gjullin & Isaak 1957). Colonized in the laboratory at Corvallis, Oregon, and maintained under malathion selection pressure, this Fresno strain became 50 times as malathion-resistant as a normal strain. However, it showed no cross resistance to parathion, Diazinon or 13 other organophosphorus compounds, except for a 7-fold cross-resistance to malaoxon and the diethyl analogue of malathion (Darrow & Plapp 1960). The Fresno strain thus was characterized by a specific malathion resistance.

Biochemical aspects of malathion resistance were first studied in the house fly (*Musca domestica* L.) March (1959) found the malathion-resistant Stauffer strain to degrade malaoxon *in vitro* more rapidly than the normal. Similar results were obtained with the Savannah (Bethesda-45) malathion-resistant strain by Oppenoorth (1959), and the detoxication was considered to be due to enzyme activity (Oppenoorth & van Asperen 1960) similar to the "phosphatase" degrading parathion and Diazinon in strains resistant to these compounds (van Asperen & Oppenoorth 1960). The increased detoxifying ability and malathion resistance was associated with an abnormally low content of aliesterase, and these characters were proved to be genetically inseparable through a series of backcrosses with the normal strain (Oppenoorth 1959). It was concluded that the malathion-resistant allele was simultaneously responsible for increased phosphatase and decreased aliesterase; the allele for Diazinon resistance also resulted in decreased aliesterase and increased detoxifying enzyme, and was due to a separate allele at the same locus (Oppenoorth & van Asperen 1960).

Meanwhile Krueger & O'Brien (1959) had established that normal house flies metabolized malathion mainly by phosphatase enzymes, whereas in *Blattella* and *Periplaneta* carboxyesterase equalled the phosphatases as hydrolytic agents. The experiments of March (1959) had established that there was no difference between malathion-resistant and normal house flies in the cuticular absorption of malathion and its conversion to malaoxon. Oppenoorth (1958) had already reported that parathion-resistant house flies did not differ from the normal in the absorption and oxidation of parathion, while Plapp & coworkers (1961) have recently found that parathion and para-oxon are more rapidly detoxified *in vivo* by parathion-resistant than by normal house flies. Whereas the mechanism of resistance to organophosphorus insecticides may be complex (Mengle & Casida 1960), nevertheless the balance of evidence at present indicates that malathion resistance in house flies would most probably derive from an increase in phosphatase activity acting particularly on malaoxon.

The first published study on malathion resistance in

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[2] Acknowledgment is made to the following for making this research possible: to A. W. Lindquist, G. W. Eddy and D. I. Darrow for kindly supplying the mosquito strains; to R. D. O'Brien and E. Y. Spencer for their advice and help in the chemistry and radioactive synthesis; and to R.W. Young and S. Debreuil for supplying the pure malathion, malaoxon and metabolites. Financial support for the investigation was provided by the National Research Council of Canada and the World Health Organization.

Culex tarsalis (Lewallen & Nicholson 1959) reported that malathion was completely converted to malaoxon in the tissues of both the Fresno-resistant and normal larvae. Darrow & Plapp (1960) exposed larvae of the Fresno resistant and the Corvallis susceptible strains in water treated with radioactive malathion at 0.05 and 1.0 p.p.m., and analyzed the water 24 hours later; they found that both strains were alike in converting 5% to 10% of the malathion to water-soluble metabolites, of which two-thirds were carboxyesterase products and one-third phosphatase products. In the present study here reported, attention was paid both to larval tissues and to excretory water, in the hope that an interstrain difference could be discovered in the carboxyesterase or the phosphatase activity as detoxifying agents of malathion or malaoxon (fig. 1).

MATERIAL.—The malathion-resistant strain of *Culex tarsalis* had been collected in Fresno County, California in 1956 and maintained in the U. S. Department of Agriculture Laboratory at Corvallis, Oregon with periodic selection with 1 p.p.m. malathion. It was colonized in our laboratory in October 1959 and maintained without further selection. The normal susceptible strain employed for comparison was the Corvallis laboratory strain.

Larvae were reared in 30-cm. enamel washbasins and fed every 3 to 4 days with 200 mg. of a mixture of 1 part ribonucleic acid (RNA), 5 parts brewer's yeast powder, and 2 parts blood albumin. Adults were kept at 23° C. and 60% to 70% relative humidity in 10-cu. ft. screened cages provided with cotton swabs soaked in 10% sucrose solution, and were given blood meals from white mice suspended in the cages overnight twice weekly. To ensure copulation, the rearing room was illuminated during the day by daylight bulbs, and by means of time switches (Paragon 3001) the illumination was progressively reduced to darkness every evening between 7 and 8 p.m., and increased again every morning between 7 and 8 a.m. (Brennan & Harwood 1953). For genetic experiments, individual adults of the required sex and strain were obtained by isolating pupae in individual shell vials containing moist cottonwool and stoppered with dry cottonwool. Individual broods from single females were obtained from eggrafts reared separately in individual washbasins.

Purified grades of malathion and malaoxon were provided by the American Cyanamid Company, Stamford, Connecticut; this firm also provided pure samples of possible metabolites of these insecticides. A purified grade of EPN was provided by E. I. du Pont de Nemours & Co., Wilmington, Delaware.

Radioactive malathion was synthesized from P^{32} phosphoric acid according to the method of Krueger & O'Brien (1959). The crude product was purified by column chromatography through adsorption alumina (Seume & O'Brien 1960). The purity of the final product was confirmed by two methods of paper chromatography (Menn et al. 1957, Lewallen & Nicholson 1959) and infrared spectroscopy.

METHODS.—Susceptibility levels of the strains and their hybrids to malathion or malaoxon were determined for almost full-grown larvae by the WHO standard method (World Health Organization 1960).

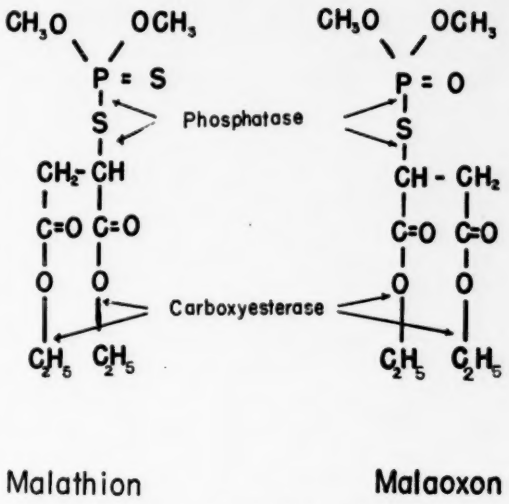

FIG. 1.—Points of enzymic hydrolysis of malathion and malaoxon.

Exposure Methods.—Experiments on metabolism *in vivo* were performed with 25 almost-full-grown larvae exposed to the insecticide at 23° C. for the desired number of hours in 25 ml. water in glass beakers, to which the required doses of malathion and malaoxon had been added in 0.1 ml. ethanol. After exposure for the desired number of hours, the larvae were removed, rinsed in water, and homogenized with a Potter-Elvehjem glass homogenizer. Each lot of 25 larvae weighed from 50 to 60 mg.

Experiments on metabolism *in vitro* were performed with 1.0 ml. enzyme source and 0.5 ml. insecticide source, incubated in test tubes at 23° C. for the desired period. The enzyme source was obtained by homogenizing 100 mg. of mature larvae with 5 ml. distilled water at 0° C. The insecticide source was obtained by dissolving the required amount of malathion or malaoxon in 1 ml. ethanol and diluting it with 249 ml. distilled water to form a fine suspension. Thus the system was unbuffered at the pH of 7.0 characteristic of the larval tissues. When oxidative metabolism of malathion was investigated, the homogenate was enriched with a mixture of co-factors (O'Brien 1957); these were added in 0.1-ml. solution to 0.9 ml. enzyme source and 0.5 ml. insecticide source, so that their final concentration in the system became 0.018M Mg ions, 0.0015M DPN and 0.01M nicotinamide.

Enzyme Assay of Malaoxon.—For the assessment of nonradioactive malaoxon, experiments *in vitro* or *in vivo* were terminated by boiling the system or the homogenized larvae respectively for 3 minutes, in order to destroy the larval enzymes. The malaoxon content was determined by enzyme assay with a cholinesterase preparation from brain tissue of *Periplaneta americana* homogenized in distilled water at a concentration of 20 mg. per ml. Aliquots of 0.5 ml. of the system or homogenized larvae to be assayed were added to 1 ml. of the cholinesterase preparation and 0.5 ml. 0.008M acetylcholine bromide; the mixture was incubated in a test tube at 37° C. for 1 hour, and the enzyme assay was stopped by the addition of 1

ml. of 20% trichloroacetic acid (TCA). The amount of acetylcholine remaining unhydrolyzed was assessed, after neutralization with 0.1 ml. 3.5N NaOH and centrifugation until clear, by the colorimetric method of Hestrin (1949) at 540 mμ wavelength. Maximum hydrolysis of ACh (i.e., 0% inhibition of ChE) was obtained when the system to be assayed was boiled at its zero time, before any malathion had been oxidized to malaoxon. Minimum hydrolysis of ACh (100% inhibition of ChE) was obtained when the enzyme assay system was treated with TCA at its zero time.

Radio Assay Methods.—For the assessment of radioactive malaoxon and malathion, experiments *in vitro* were terminated by adding 23.5 ml. 0.2% TCA to the 1.5-ml. system, and experiments *in vivo* were terminated by homogenizing the larvae in 0.2% TCA; the denaturation of water-soluble proteins with TCA is necessary for thorough extraction of these insecticides (Matsumura 1959). The sample, now at pH 2.0, was neutralized to pH 7.0 with approximately 0.1 ml. N KOH, and the malathion and malaoxon were extracted by shaking it with an equal volume of chloroform in two successive extractions; this was found to be 99.7% efficient.

The radioactive materials not chloroform-extractable at pH 7.0 were taken to comprise the water-soluble metabolites of malathion and malaoxon. Of these, the monocarboxylic esters and dicarboxylic acids produced by the action of carboxyesterase, being weak acids, would be extractable from water into chloroform (Darrow & Plapp 1960) or ether (O'Brien 1960) at pH 2.0. Therefore the carboxyesterase products were extracted by adding a Coleman standard buffer tablet at pH 2.0 to the aqueous extract and shaking it with two successive equal volumes of diethyl ether. The metabolites produced by the action of phosphatases, which split off the succinic acid sidechain of these insecticides, are sufficiently strong acids to remain in the aqueous phase after chloroform extraction at pH 7.0 and ether extraction at pH 2.0.

The radioactive malathion and malaoxon in the chloroform extract were separated from each other by evaporating the chloroform and redissolving in n-hexane, and passing the n-hexane extract through an alumina adsorption column (Krueger & O'Brien 1959, Seume & O'Brien 1960). Columns were freshly prepared each time, being packed with adsorption alumina containing 3% water by weight and suspended in dry n-hexane, and then saturated with nonradioactive malathion by passing 50 ml. of a 1 p.p.m. solution in n-hexane through the column. The extract containing radioactive malathion and malaoxon was added to the column, being preceded and followed by washing with 50 ml. n-hexane. Then the malathion was eluted with 150 ml. benzene, and finally the malaoxon was eluted with 200 ml. chloroform. The total recovery of radioactivity was 60% to 80%; therefore the values obtained for the malathion and malaoxon samples were equally adjusted upwards to compensate for this deficiency.

The water-soluble metabolites of radioactive malathion were separated individually by passage through an ion-exchange column (Plapp & Casida 1958). The column was filled with the sulfonated polystyrene resin Dowex 1×8 (100–200 mesh), which had been thoroughly washed with 50 ml. HCl diluted to pH 3.0. The sample, at the unbuffered pH of 7.0, from which malathion and malaoxon had been removed by choloform extraction, was poured into the column. The individual metabolites were then eluted with a series of eluents in which the pH was progressively increased and the water progressively replaced by ethanol and later acetone (Krueger & O'Brien 1959). The successive peaks of radioactivity were identified by comparison with the successive peaks of P content obtained by cochromatography in a separate similar column of nonradioactive pure samples of possible metabolites.

Radioactive samples were assessed by means of a liquid scintillation well counter (Tracerlab SC-57) connected to a scaler (Tracerlab SC-33B). The 5-ml. samples were held in glass colorimetric tubes with a diameter of 1.2 cm. and a wall thickness of 1 mm., placed in the well counter above the gamma-crystal detector; although P^{32} produces beta radiation, it may be assessed with little loss and no inaccuracy by a gamma crystal. Assessments consisted of five successive counts of 3 minutes' duration; the voltage was adjusted to 1200 v., and the sensitivity to a background count of 130 to 140 c.p.m.

Determination of Enzyme Activity.—Cholinesterase activity was assessed by homogenizing 50 mg. of larvae in 5 ml. distilled water and incubating 1 ml. of the homogenate with 0.5 ml. 0.008M acetylcholine bromide solution for 1 hour at 37° C. and pH 7.0. The amount of acetylcholine remaining unhydrolysed was then determined by the Hestrin test. Sensitivity of the cholinesterase to inhibition was determined by adding 0.5 ml. of suspensions of malaoxon (0.04 to 1.0 p.p.m.) or malathion (100 to 400 p.p.m.).

Aliesterase activity was assessed by a slight modification of the method described by Oppenoorth (1959). Approximately 400 mg. of larvae were homogenized in 23 ml. 1% NaCl at 0° C., and 2.3-ml. aliquots were placed in the moats of Warburg flasks kept in a water bath at 37° C. The substrate was methyl n-butyrate, 0.4-ml. aliquots of a 5.7% emulsion in 1.26% aqueous $NaHCO_3$ being placed in the side arms. After gassing with a mixture of 5% CO_2 and 95% N_2, and subsequent temperature equilibration, the substrate was tipped into the moat, and the CO_2 produced was read from the manometers at 10-minute intervals for 50 minutes.

Carboxyesterase activity in the various cellular fractions was assessed by differential centrifugation (Gonda et al. 1957) of a larval homogenate in 0.25M sucrose (100 mg. per 5 ml.) at 0° C. in a refrigerated centrifuge (Servall RC-2 with SS-34 rotor). The debris and nuclei were first removed by centrifuging for 5 minutes at 2,500 r.p.m. (770×g). Then the mitochondrial fraction was separated by centrifuging for 10 minutes at 8,500 r.p.m. (8500×g); it was resuspended in 5 ml. ice-cold 0.25M sucrose, and 1 ml. of this suspension was assessed for carboxyesterase activity. Finally the microsome fraction was separated by centrifuging for 1 hour at 17,000 r.p.m. (34,800×g); it was similarly resuspended in sucrose solution, and compared for carboxyesterase activity with the supernatant containing the soluble fraction.

RESULTS.—Tests of the third- to fourth-stage larvae of either strain with malathion (table 1) showed the Fresno strain was 45 times as malathion-resistant as the Corvallis strain. Tests with malaoxon showed the resistant strain to be nine times as malaoxon-resistant as the susceptible

Table 1.—Insecticide susceptibility and esterase levels of Corvallis susceptible and Fresno malathion-resistant strains of *Culex tarsalis*.

		SUSCEPTIBLE	RESISTANT
Malathion resistance	LC_{50} in p.p.m., Oct. 1959	0.05	2.25
	Jan. 1960	0.03	1.2
Malaoxon resistance	Oct. 1959	0.075	0.66
Cholinesterase activity	% ACh hydrolysed per hour	38.6	38.3
Cholinesterase sensitivity	I_{50} for malathion	1.2×10^{-4}M	1.2×10^{-4}M
	I_{50} for malaoxon	3.7×10^{-8}M	3.8×10^{-8}M
Aliesterase activity	cu. mm. CO_2/g./min.	7.9±3.3	9.1±2.0

strain. The cholinesterase activity of both strains proved to be identical, and so was the sensitivity of the ChE of either strain to inhibition by malathion and malaoxon. For both strains, malaoxon showed approximately 2,000 times as much anticholinesterase activity as malathion. There was no significant difference in the aliesterase activity of the two strains.

Activation of Malathion to Malaoxon.—Larvae of the resistant and susceptible strains were exposed to 1 p.p.m. malathion for 30 minutes and then assessed for malaoxon content by enzyme assay with cockroach ChE. The results (table 2) showed that the resistant larvae came to contain only one-third as much malaoxon as the susceptible, the difference being significant ($t_9=2.29$). In 5 p.p.m. malathion, which paralyzed some of the susceptible larvae, the difference shown between the average figures was not significant ($t_9=1.02$); while in 25 p.p.m. malathion, which killed 95% of the susceptibles and even 13% of the resistants, the susceptible larvae came to contain no more malaoxon than the resistants, probably because death had inhibited their oxidative capacity.

Larvae of either strain were then exposed to 5 p.p.m. of radioactive malathion, and the absorption of malathion and production of malaoxon was followed for 4 hours (table 3). The malaoxon radio-assay figures indicate that the resistant strain comes to contain less malaoxon than the susceptible. The malathion radioassays indicate that the resistant strain absorbs malathion at the same rate as the susceptible. The results at 5 and 27 p.p.m. malathion indicate that the internal malathion level reaches equilibrium after about 1 hour of exposure.

Larval homogenates of either strain were incubated at 23° C. with added co-factors in 27 p.p.m. malathion (40 μg. per 1.5-ml. system). There was no significant difference between the strains in the amounts of malaoxon found by bioassay at hourly intervals in the 6-hour incubation period, at the end of which 0.16 to 0.50 μg. malaoxon were present. When the experiment was repeated in 0.66 p.p.m. radioactive malathion, there was again no interstrain difference in the malaoxon produced during a 4-hour period, which resulted in 0.03 to 0.07 μg. malaoxon being present in each digest.

The results indicate that there is no difference between the strains in the rate of activation of malathion to malaoxon. Nevertheless the malaoxon content is less in the resistant strain. Since there is no interstrain difference in the absorption rate of malathion, the characteristic of the resistant strain must be sought in an enhanced detoxication of the malaoxon produced.

Fate of Malaoxon.—Larvae of resistant and susceptible strains were exposed to 1 p.p.m. malaoxon, and their content of this toxicant was enzyme assayed at hourly intervals. The results (table 4) indicate that the malaoxon content, though identical initially, came to be considerably less in the resistant than in the susceptible strain. Since the issue was complicated by mortality of the susceptible larvae (for which the LT_{50} was 3 hours), the experiment was repeated with a 1.5-hour exposure followed by transfer to clean water; the same difference was shown, the resistant larvae at 20 hours containing less than one-third as much malaoxon as the susceptible larvae.

Larval homogenates of either strain were incubated in

Table 2.—Malaoxon content of larvae of *C. tarsalis* exposed to malathion: μg. per 25 larvae, exposed for 30 mins.[a]

STRAIN	MALATHION CONCENTRATION (P.P.M.) 1.0	5.0	25
Susceptible	0.19±0.04	0.18±0.05	0.11±0.03
Resistant	0.06±0.04	0.10±0.06	0.13±0.03
Difference/S.E. Diff.	$t_9=2.29$	$t_9=1.02$	$t_9=0.48$

[a] Average of five replicates each; anticholinesterase assay.

Table 3.—Malaoxon content of larvae of *C. tarsalis* exposed to radioactive malathion: μg. per 25 larvae, radioassay.

STRAIN	MALATHION CONCENTRATION (P.P.M.)	HOURS OF EXPOSURE 0.5	1	2	3	4
Malathion absorbed						
Susceptible	27	4.5	5.5	—	10.0	8.0
Resistant	27	0.5	11.0	10.0	9.5	10.5
Susceptible	5	—	6.0	6.5	6.0	7.0
Resistant	5	—	7.0	5.5	6.0	7.5
Malaoxon produced						
Susceptible	27	1.0	0.5	—	2.0	0.05
Resistant	27	0	0	0	1.5	0.05
Susceptible	5	—	2.5	2.5	2.5	2.0
Resistant	5	—	2.5	1.0	1.5	0.5

Table 4.—Malaoxon content of larvae exposed to 1 p.p.m. malaoxon: μg. per 25 larvae, anticholinesterase assay.[a]

STRAIN	DURATION OF EXPOSURE	HOURS AFTER BEGINNING OF EXPOSURE 1	2	3	4	5	20
Susceptible	continuous	0.19	0.22	0.25	0.34	0.50	—
Resistant	continuous	0.18	0.18	0.17	0.21	0.22	—
Susceptible	1.5 hours	0.18	0.15	0.23	0.16	0.16	0.21
Resistant	1.5 hours	0.18	0.18	0.10	0.15	0.12	0.06

[a] In these experiments, unlike the others, the larval ChE was not destroyed before enzyme assay.

Table 5.—Malaoxon content of homogenates incubated with malaoxon: μg. left unhydrolyzed by 20 mg. larval tissue, anticholinesterase assay.

Strain	Hours of Incubation		
	Zero	0.5	3.0
Susceptible	4.00	3.72±0.24	2.66±0.44
Resistant	4.00	2.65±0.46	2.11±0.35
Difference/S.E. Diff.		t_7=2.06	t_{13}=1.00

2.67 p.p.m. malaoxon (4 μg. per 1.5-ml. system), and the malaoxon remaining undegraded at 30 minutes and 3 hours was assessed by enzyme assay. The results (table 5) indicate that malaoxon is destroyed faster by the resistant homogenates, the difference at the 0.5-hour period being almost significant at the 5% level (t_7=2.06). This difference could account for the modest malaoxon resistance of the Fresno strain, which is about nine times the normal. To explain the much higher (45-fold) malathion resistance of this strain, the difference must therefore be sought in the enhanced degradation of malathion as well as malaoxon.

Fate of Malathion in Vivo.—Larvae of the resistant and susceptible strains were exposed to radioactive malathion for 30 minutes, and were then radio assayed for chloroform-extractable material (mainly malathion plus some malaoxon) and for water-soluble material (fractionated into phosphatase products and carboxyesterase products). The water in which they were exposed (25 larvae in 25 ml.) was similarly assayed. This fractionation accounted for at least 99.75% of the radioactive malathion added. Exposures of larvae were made at 1 p.p.m. malathion with four replicates, and at 5 p.p.m. malathion with seven replicates. The radioactive malathion in itself, on exposure to water for 30 minutes, showed only 0.36% of the radioactivity as water-soluble (0.04% in the phosphatase and 0.32% in the carboxyesterase fractions).

The results (table 6) indicate that there was no significant difference between the strains in malathion metabolism or metabolite production. Both strains were alike in metabolizing 2% to 8% of the ambient malathion, and in showing 60% to 80% of the metabolites in the surrounding water rather than the larval bodies. In order to eliminate malaoxon as a source of these metabolites from radioactive malathion, the experiment at 5 p.p.m. was repeated with larvae previously treated with 0.3 p.p.m. nonradioactive malaoxon for 1 hour, so that the production of radioactive malaoxon would be largely blocked. This had the effect of reducing the production of water-soluble metabolites by about one-half; but no interstrain differences appeared.

Table 7.—Malathion and metabolites in larvae of *C. tarsalis* cleaned after exposure to radioactive malathion.[a]

Strain	Solvent-Extractable	Phosphatase Products	Carboxyesterase Products
Percentage in larval bodies			
Susceptible	0.79± .21	0.81± .44	0.52± .30
Resistant	0.17± .01	0.59± .27	1.00± .25
Difference/S.E. Diff.	t_7=2.75	t_7=0.43	t_7=1.25
Percentage in cleaning water			
Susceptible	0.39+ .22	0.44± .27	0.30± .13
Resistant	0.19± .13	0.46± .16	0.18± .18
Difference/S.E. Diff.	t_7=0.80	t_7=0.06	t_7=0.54

[a] 25 larvae exposed to 25 μg. radioactive malathion in 25 ml. water for 30 mins., then removed to 25 ml. distilled water for 30 minutes.
Amounts expressed as percentage of total malathion applied; radio-assay.

The large amount of water-soluble metabolites produced in 30 minutes suggested that extracorporeal hydrolysis might take place. Therefore 125 μg. radioactive malathion was added to 25 ml. water in which 25 larvae of either strain had been temporarily placed for the previous 30 minutes; the amounts of water-soluble metabolites produced from malathion by this contaminated water were assessed 30 minutes later. Replicated experiments indicated that no carboxyesterase products were thus produced by the larval excreta, but with both strains the amount of phosphatase products was raised from 0.05% (control) up to 0.34% to 0.38%. It is thus evident that much of the phosphatase products are produced by extracorporeal hydrolysis, probably by enzymes of the alimentary canal.

Since the essential interstrain difference should be sought in the metabolism of malathion in the tissues of the body close to the vital sites, larvae of either strain were exposed to 1 p.p.m. radioactive malathion for 30 minutes, and then transferred to clean water for a subsequent 30 minutes for ridding their gut of the extracorporeal material; they were then assessed for tissue content of malathion and metabolites. These conditions of exposure involved no knockdown of the susceptible strain. The

Table 6.—Malathion and metabolites in larvae of *C. tarsalis* and ambient water.[a]

Strain	Malathion Concentration (p.p.m.)	Larval Bodies			Ambient Water		
		Solvent-Extractable	Phosphatase Products	Carboxyesterase Products	Solvent-Extractable	Phosphatase Products	Carboxyesterase Products
Susceptible	1	1.71±.32	1.55±.09	1.44±.07	90.29±.38	1.50±.28	3.30±.22
Resistant	1	1.48±.09	1.07±.14	1.55±.13	90.82±.25	1.19±.25	3.68±.19
Susceptible	5	0.08±.02	0.13±.06	0.05±.01	97.86±.27	0.45±.12	1.37±.26
Resistant	5	0.04±.01	0.11±.04	0.07±.03	98.23±.15	0.47±.12	1.05±.08
Blocked with nonradioactive malaoxon							
Susceptible	5	0.08±.02	0.04±.03	0.01±.01	99.01±.13	0.28±.03	0.57±.17
Resistant	5	0.03±.02	0.01±.01	0.02±.02	98.95±.14	0.30±.08	0.69±.22
Control: malathion in water without larvae							
—	5	—	—	—	99.65	0.32	0.04

[a] Amounts expressed as percentage of total radioactive malathion applied by exposure of 25 larvae in 25 ml. water for 30 mins.; radio-assay.

Table 8.—Water soluble metabolites produced by larvae of *C. tarsalis* exposed to radioactive malathion.[a]

STRAIN	$(OH)_2P(O)OH$ PEAK 1	$(MeO)_2P(O)OH$ PEAK 2	$(MeO)_2P(O)SH$ PEAK 6	$(MeO)_2P(S)OH$ PEAK 7	$(MeO)_2P(S)SH$ PEAK 10	TOTAL PHOSPHATASE PRODUCTS	MONO ESTER PEAK 4	DIACID PEAK 5	TOTAL CARBOXYESTERASE PRODUCTS	PEAK 3	PEAK 8	PEAK 9	TOTAL UNKNOWNS
Susceptible	8.6	6.8	19.4	11.1	2.8	48.9	28.8	8.9	37.7	5.8	3.2	2.9	11.9
Resistant	17.1	14.8	11.7	5.3	5.9	54.8	23.2	11.8	35.0	1.0	4.1	2.6	7.7

[a] 400 mg. larvae exposed to 5 p.p.m. radioactive malathion in 25 ml. water for 30 minutes. Aqueous extract of larvae combined with ambient water before chromatography.

Amounts expressed as percentage of the total recovered (35 μg. for susceptible, 49 μg. for resistant); radioassay.

results (table 7) show no interstrain difference in phosphatase products, but the amount of carboxyesterase products in the resistant strain was on the average about twice that in the tissues of the susceptible larvae, although the difference was not quite significant. The outstanding interstrain difference was in the tissue content of solvent-extractable radioactivity (malathion and malaoxon), which was almost five times as great in the susceptible as in the resistant strain, the difference being significant at the 3% level of probability.

Chemical Nature of Metabolites.—Larvae of either strain were exposed to 5 p.p.m. radioactive malathion for 30 minutes, and the combined aqueous extracts of larvae and the ambient water were passed through an ion-exchange column; the successive metabolites were discriminated by peaks in radioactivity of the eluate. The results (table 8) are shown in the same form as those displayed for other insects by Krueger & O'Brien (1959), with the exception that their unknown peak 6 has been identified as dimethyl phosphorothiolate. This compound, which would be expected to isomerize with dimethyl phosphorothionate (peak 7) was the most abundant phosphatase product in the susceptible strain; whereas phosphoric acid itself was the most abundant phosphatase product in the resistant strain. Among the carboxyesterase products, the monocarboxylic esters were twice as abundant as the dicarboxylic acids, and constituted about half of all the metabolites. This distribution of metabolites was confirmed qualitatively by paper chromatography either with n-butanol as the mobile phase (Menn et al. 1957) or with aqueous propionic acid as the mobile phase (Lewallen & Nicholson 1959). The latter method gave a clear separation and identification of malaoxon as an important metabolite in the solvent-extractable fraction.

Malathion Degradation in Vitro.—Larval homogenates of either strain were incubated in 333 p.p.m. radioactive malathion (500 μg. per 1.5-ml. system) at 23° C. and pH 7.0 for 3 hours, and the amounts degraded into the two classes of water-soluble products were assessed. The results of quadruplicate experiments (table 9) show that the homogenates of the resistant strain produce 2 to 3 times as much phosphatase and carboxyesterase products as the susceptible strain, the interstrain differences being highly significant. When the homogenates were incubated under anaerobic conditions, the interstrain difference in phosphatase products disappeared, suggesting that malaoxon is the main substrate for the phosphatase hydrolysis. This view was strengthened by duplicate experiments in which addition of 50 μg. of nonradioactive malaoxon was found to reduce the phosphatase products to the very low figures of 1.8% for susceptible and 0.6% for resistant homogenates. On the other hand, neither anaerobic conditions nor malaoxon blocking and competition affected the production of carboxyesterase products, of which the resistant strain produced 2 to 3 times more than the susceptible strain. Addition of 0.0002 μg. of EPN, a strong inhibitor of carboxyesterase in mammals (Seume & O'Brien 1960), reduced the interstrain difference in carboxyesterase activity to insignificance.

Larval homogenates were fractionated by differential centrifugation into mitochondria, microsomes and soluble fraction, and tested for carboxyesterase activity at 23° C. and pH 7.0. With resistant homogenates, it was found that three times as much carboxyesterase activity was in the mitochondrial fraction as in the soluble fraction, and six times as much as in the microsome fraction. The phosphatase activity was found to have a similar distribution. Therefore enzyme preparations were made of the mitochondrial fraction of either strain and tested for carboxyesterase activity by incubation at 23° C. for 30 minutes at the unbuffered pH of 7.0, and at buffered

Table 9.—Water-soluble metabolites produced by homogenates incubated with radioactive malathion: amounts expressed in percentage of total malathion in system;[a] radioassay.

STRAIN	PHOSPHATASE PRODUCTS	CARBOXYESTERASE PRODUCTS
Aerobic conditions		
Susceptible	6.3±1.5	9.6±2.3
Resistant	14.4±1.4	24.3±0.3
Difference/S.E. Diff.	$t_7=3.90$	$t_7=6.28$
Anaerobic conditions		
Susceptible	6.2±1.7	5.3±1.7
Resistant	8.3±2.4	15.2±3.2
Difference/S.E. Diff.	$t_7=0.71$	$t_9=2.75$
Aerobic conditions plus EPN		
Susceptible	6.1±2.5	4.8±3.6
Resistant	5.7±2.6	11.6±4.3
Difference/S.E. Diff.	$t_7=0.11$	$t_7=1.21$

[a] 20 mg. larval tissue, 1.5 ml. water, 500 μg. radioactive malathion; incubated at 23° C. for 3 hours. Non-enzymic control figures not subtracted.

Table 10.—Carboxyesterase activity in mitochondrial fraction of homogenates: amounts expressed as percentage of total radioactive malathion in system;[a] radio-assay.

Strain	pH	Phosphatase Products	Carboxyesterase Products	$t_{\frac{1}{2}}$ for Carboxyesterase
Susceptible	6.0	0.9±.3	0.1±.1	3.44
Resistant		0.8±.3	2.5±.7	
Susceptible	7.0	1.8±.6	1.7±.6	1.34
Resistant		2.1±.1	2.6±.3	
Susceptible	7.0 (unbuffered)	—	0.5±.3	5.0
Resistant		—	3.4±.5	
Susceptible	8.0	0.6±.2	0.9±.4	1.24
Resistant		1.0±.4	1.6±.4	

[a] Mitochondrial fraction from 20 mg. larvae, 1.5 ml. water, 250 μg. radioactive malathion, incubated at 23° C. for 3 hours.

(0.1M Coleman standard) pH values between 6.0 and 8.0. The results (table 10) show that a mitochondrial fraction from the resistant strain contains much more carboxyesterase activity than the susceptible strain, the difference being greater at the lower pH values.

Larval homogenates of either strain were tested for carboxyesterase activity at different substrate concentrations of malathion. The figures for 30-minute production of water-soluble metabolites were plotted to obtain the Michaelis constant, according to the method described by Dixon & Webb (1958). They show (fig. 2) that the Michaelis constant for the enzyme activity is identical in either strain, namely 0.11×10^{-4}M, indicating that the difference in carboxyesterase activity between the resistant and susceptible strains is quantitative rather than qualitative. They also are suggestive that the lower activity in the susceptible strain is due to noncompetitive inhibition.

Detoxifying-Enzyme Inhibitors as Synergists for Malathion.—The relative importance of carboxyesterase and phosphatase content as defense mechanisms was investigated by assessing the synergistic effect *in vivo* of compounds known to be inhibitors of one or another of the enzymes. In addition to the carboxyesterase inhibitor EPN, sodium fluoride is known to be an inhibitor for acid phosphatase in mosquitoes (Lambremont 1959), and was found to inhibit 70% of the phosphatase activity *in vitro* and 90% *in vivo* in our strains of *C. tarsalis*. The phosphatase inhibitor NaF showed no synergism for malathion against the susceptible strain and only slight synergism against the resistant strain (table 11); on the other hand the carboxyesterase inhibitor EPN was highly synergistic for malathion against the resistant strain, but not the susceptible. EPN showed only slight synergism for malaoxon. These results therefore confirm the idea that carboxyesterase is the most important characteristic for the strong resistance to malathion, but not for the modest resistance to malaoxon.

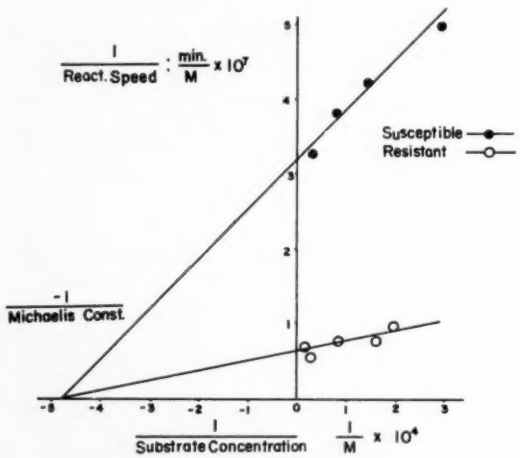

FIG. 2.—Carboxyesterase activity as related to malathion concentration in homogenates from susceptible and resistant strains.

Table 11.—Effect of enzyme inhibitors[a] on susceptibility of larvae of *C. tarsalis* to malathion and malaoxon: LC_{50} in p.p.m.

Toxicant	Strain	No Inhibitor	NaF 1.0 p.p.m.	EPN 0.2 p.p.m.
Malathion	Susceptible	0.051	0.074	0.050
	Resistant	2.50	1.61	0.625
Malaoxon	Susceptible	0.075	0.063	0.025
	Resistant	0.66	0.28	0.18

[a] Added in constant concentration to the graded concentrations of malathion and malaoxon employed in the susceptibility test.

Increased Carboxyesterase Activity as the Cause of Malathion Resistance.—Proof that increased carboxyesterase activity is the effective mechanism for malathion resistance would be supplied if the higher enzyme content is inherited as an inseparable concomitant of the resistance. To determine whether the high carboxyesterase content of the Fresno strain is genetically congruent with the malathion resistance, and not an accident associated with its geographical origin, this strain was crossed with the susceptible Corvallis strain; the F_1 hybrid offspring were successively backcrossed with the susceptible strain for two further generations, so that the genome would become increasingly of the Corvallis type (fig. 3). At each successive generation of hybrids, the full-grown larvae were selected by exposure to 1 p.p.m. malathion for 24 hours, which eliminated the susceptible homozygotes but preserved the heterozygotes with the malathion-resistant allele from Fresno.

Larvae of each of these backcross generations (B_1, B_2) were assayed for carboxyesterase activity and malathion resistance; of course no selection pressure had been applied to them before assay. The results (table 12) represent the average values for reciprocal crosses, which did not differ significantly from each other. It is seen that despite three generations of backcrossing with the Corvallis strain, the malathion resistance maintained by selection pressure was still accompanied by a high level of carboxyesterase activity. A somewhat similar result was obtained by a program of backcrossing and selection with 0.25 p.p.m. malaoxon, except that the carboxyesterase level did not stay up as the phosphatase level did. It is

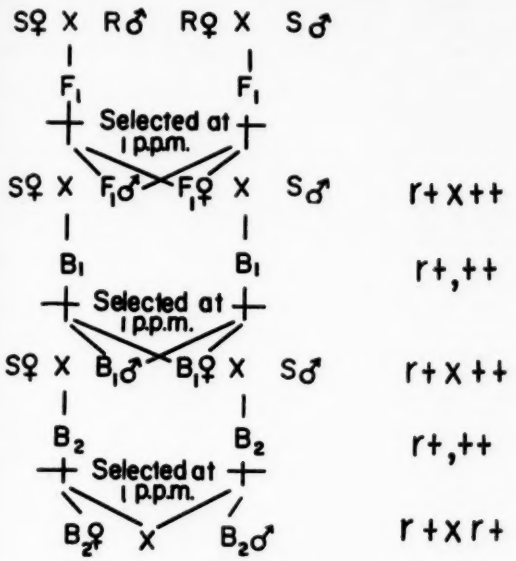

FIG. 3.—System of backcrossing with the susceptible strain and selection to preserve the resistance gene allele.

however clear that malathion resistance is genetically inseparable from increased carboxyesterase activity.

Genetics of Malathion Resistance.—When males of the Fresno strain were crossed with females of the susceptible strain, the F_1 hybrid larvae showed a steep dosage-mortality line with an LC_{50} of 0.7 p.p.m. malathion (fig. 4). When four females were taken from the Fresno colony and crossed with susceptible males, the F_1 offspring segregated into two equal classes, one of the hybrid type and the other similar to the susceptible type. Within the dosage range from 0.06 to 0.5 p.p.m. malathion, all larvae of the susceptible type are killed, but none of the hybrid type. It is clear that the Fresno females taken for the crosses had been heterozygotes, while the males had been homozygotes for the malathion resistance.

Therefore a subcolony was taken from the Fresno strain

Table 12.—Carboxyesterase activity and malathion resistance of successive generations of *C. tarsalis* in the back-cross-and-selection experiment.

GENERATION	LC_{50} (P.P.M.)	CARBOXYESTERASE[a] PRODUCTS	PHOSPHATASE[a] PRODUCTS
Malathion			
R	1.8	4.4±0.6	4.3±0.8
S	0.043	0.8±0.3	3.2±0.9
F_1	0.80	4.5±1.0	—
B_1	0.75	4.2±0.4	—
B_2	0.80	5.4±0.6	—
Malaoxon			
B_1	0.8	4.8±0.4	4.6±0.3
B_2	0.8	2.9±1.0	4.0±3.0

[a] Amount produced in test system of 250 μg. radioactive malathion in 1.5 ml. water, incubated at 23° C. for 30 minutes, expressed in per cent of total radioactivity recovered.

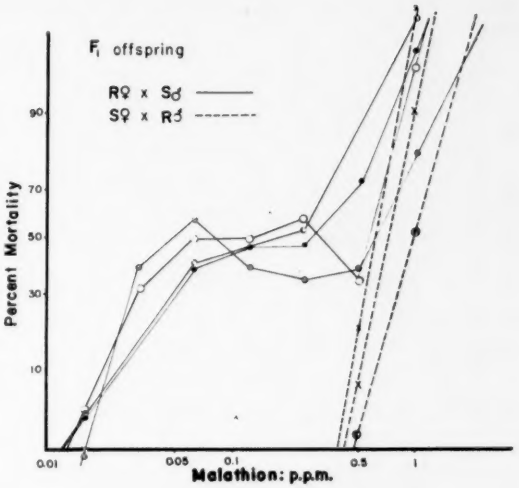

FIG. 4.—Dosage-mortality relationships of hybrid offspring from reciprocal crosses between the resistant and susceptible strains.

and selected with 2 p.p.m. malathion, which would leave only homozygotes as survivors. When females from this selected subcolony were crossed with susceptible males, the F_1 offspring showed a steep dosage-mortality line with an LC_{50} of 0.8 p.p.m. malathion (fig. 5). This line shows the heterozygotes to be clearly distinguished from the susceptible strain, and thus it is clear that the gene allele for malathion resistance is not recessive. The line for the hybrids approaches that for the resistant strain, but this material is contaminated by heterozygotes, as the previous results and the shallower slope for R_2 indicate; however the evidence is sufficient for the factor for malathion resistance to be considered more dominant than recessive.

The F_1 hybrids were selected with 1 p.p.m. to eliminate susceptible homozygotes, and the heterozygotes thus

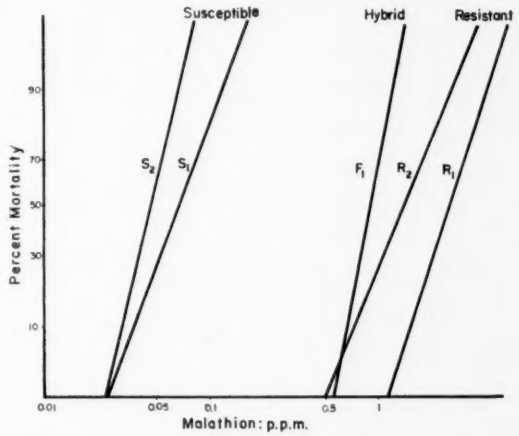

FIG. 5.—Dosage-mortality regression lines for the resistant and susceptible strains, and for hybrid offspring from homozygous-resistant females and susceptible males.

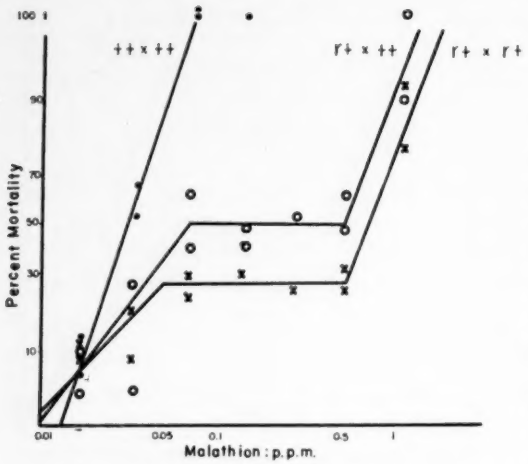

FIG. 6.—Segregation of malathion-resistance in the F_2 offspring. Single-brood data from crosses of ++×++ (all susceptible), r+×++(1:1 segregation) and r+×r+ (3:1 segregation). Points taken from reciprocal crosses of each type.

surviving were backcrossed with the susceptible strain. The backcross offspring thus obtained (fig. 6) clearly showed 1:1 segregation into two types, corresponding to susceptible homozygotes and partially resistant heterozygotes. That malathion resistance is due to a single genetic factor is also indicated in the F_2 data obtained from crosses between F_1 individuals of different types. The nine F_2 broods tested fell into three classes (fig. 7): those in which all were susceptible; those showing 1:1 segregation into susceptible and resistant (heterozygotes); and those showing 1:3 segregation into susceptible and resistant (heterozygotes and homozygotes).

DISCUSSION.—It is evident that the effective toxicant in the action of malathion on larvae of *Culex tarsalis* is malaoxon. The cholinesterase present in equal quantities in either strain studied was 2,000 times as sensitive to malaoxon as to malathion. Incubation of malathion with larval tissues *in vivo* and *in vitro* resulted in the development of a metabolite with strong anticholinesterase activity as shown by enzyme assay with cockroach ChE. Malaoxon was identified as an initial metabolite of malathion by paper chromatography, and alumina-column chromatography of the solvent-extractable fraction present in larvae exposed to radioactive malathion showed that 10% to 30% of the insecticide absorbed had been transformed into malaoxon. Malathion is absorbed at a similar rate in either strain, and it appears that it is oxidized to malaoxon at equal rates. The amounts of malaoxon accumulated in these mosquito larvae in 30 minutes, corresponding to a tissue concentration of approximately 1.0 μg./g., is appreciably higher than the amounts found by Krueger & O'Brien (1959) in the house fly, which were approximately 0.2 μg./g.

When larvae of either strain are exposed to equal concentrations of malathion, the resistant strain comes to contain about one-third as much malaoxon as the normal strain. This difference is partly caused by its greater ability to detoxify malaoxon; for when larvae are exposed to malaoxon, this toxicant is eliminated by the resistant but not the susceptible strain, and homogenates of resistant larvae destroy malaoxon almost five times as fast as those of susceptible larvae.

However since the malathion resistance of the Fresno strain is much more pronounced than its malaoxon resistance, it is logical to look for interstrain differences in the rate of degradation of malathion into nontoxic water-soluble metabolites. This proved difficult to put in evidence, since much of the degradation takes place in the gut and thus really outside the body, and indeed phosphatase hydrolysis persists even in the surrounding water. Of the water-soluble metabolites, which come to be much more abundant in the ambient water than in the larval bodies, some two-thirds are carboxyesterase products, as Darrow & Plapp (1960) had found. Only when larvae were cleared of their gut contents by a period in clean water could an interstrain difference be shown in the larval tissues themselves; under these conditions the resistant strain proved to contain about twice as much carboxyesterase metabolites and correspondingly less of the insecticide itself.

The interstrain difference was clearly shown *in vitro*, where homogenates of resistant larvae were 2 to 3 times as active as those of susceptible larvae in producing water-soluble metabolites (both carboxyesterase and phosphatase products) from radioactive malathion. Much of the substrate evidently passed through malaoxon as the initial metabolite, since anaerobic conditions of malaoxon blocking and competition resulted in a lower metabolite yield, particularly of the phosphatase products. It is therefore likely that phosphatases are primarily concerned with malaoxon metabolism and the modest malaoxon resistance. That it is the carboxyesterase activity that is the deciding factor in malathion metabolism and malathion resistance is demonstrated by experiments *in*

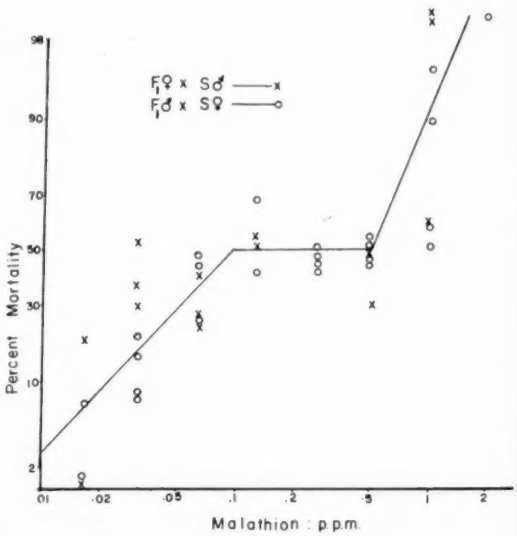

FIG. 7.—Dosage-mortality relationships of offspring of the backcross between the F_1 heterozygotes and the resistant strain.

vitro and *in vivo*, where the carboxyesterase inhibitor, EPN, proved to be a strong synergist for the resistant strain, whereas the phosphatase inhibitor, NaF, showed only a weak synergistic effect.

When the carboxyesterase activity in the mitochondrial cellular fraction was assessed, it became clear that it was many times higher in the malathion-resistant than in the susceptible strain. Since both the Michaelis constant relating rate and substrate concentration, and also the speed of inhibition by EPN as reflecting turnover rate, were identical in either strain, the interstrain difference in carboxyesterase activity is quantitative rather than qualitative. Indeed, the spectrum of different metabolites is similar in either strain, the monocarboxylic ester being the most abundant in both cases.

That the increased carboxyesterase activity is inseparable from malathion resistance, and therefore almost certainly its cause, was proved by the program of backcrossing of the Fresno with the Corvallis strain accompanied by malathion selection; the increased carboxyesterase activity remained as a concomitant of the malathion resistance even when the genome had come to be seven-eighths of Corvallis origin. The sharp segregation discovered in F_1 and F_2 offspring of hybrid crosses indicated that the character malathion resistance (and increased carboxyesterase activity) is due to a single genetic factor, the mutant allele being partially dominant. The Fresno resistant strain as maintained in the laboratory evidently contained heterozygotes as well as homozygotes for this allele.

In contrast to malathion resistance in the house fly (Oppenoorth & van Asperen 1960) where phosphatase activity is increased at the expense of aliesterase, malathion resistance in *Culex tarsalis* involves no decrease in aliesterase, and the important increase is in carboxyesterase activity. Indeed, malathion is different from other organophosphorus insecticides in that it possesses a molecular structure vulnerable to carboxyesterase hydrolysis. But both types of malathion resistance have been traced to a single gene allele which evidently induces an increase in activity of a detoxifying enzyme; with *C. tarsalis*, the Michaelis-constant data suggest that the allele may operate by eliminating some noncompetitive inhibition of the carboxyesterase. The present study constitutes the third example of a gene-enzyme-resistance relationship in insects, the first of which was discovered by Lovell & Kearns (1959) for the mode of inheritance of DDT-dehydrochlorinase and DDT resistance.

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The Effect of Controlled Temperature and Humidity on the Residual Life of Certain Insecticides[1]

G. S. KALKAT,[2] R. H. DAVIDSON,[3] and C. L. BRASS,[4] *The Ohio State University, Columbus*

ABSTRACT

The residual life of commercial emulsifiable formulations of heptachlor, aldrin, Diazinon® (*O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate), parathion, malathion, and an acetone solution of heptachlor epoxide was determined under controlled conditions of temperature and humidity, using three- to four-week-old adults of the red flour beetle, *Tribolium castaneum* (Herbst).

At 90° F. and a R.H. (relative humidity) of 55%±5%, the residual life in days of aldrin, heptachlor, heptachlor epoxide, and Diazinon was 8, 10, 28, and 99, respectively, whereas at the same R.H. but at 110° F., the residual life was 4, 4, 21, and 28 days, respectively. Parathion lost its effectiveness in 41 days at 90° F. and 55%±5% R.H. but still gave 44% mortality on the 18th day at 110° F. and 55%±5% R.H. At 90° F. and 55%±5% R.H. malathion gave 78% mortality on the 21st day, whereas, at the same R.H. but at 110° F., it gave 32% mortality on the 28th day.

Relative humidity during the time of exposure of the test insects markedly affected mortality. When assayed at a R.H. of 55%±5%, Diazinon showed a complete loss of toxicity on the 28th day when held at 110° F. and 55%±5% R.H., but it gave 100% mortality when held at similar temperature conditions but assayed at 90%±5% R.H. Likewise, parathion, aldrin, heptachlor, heptachlor epoxide, and malathion also gave higher mortality of the insects when assayed at 90%±5% R.H.

Investigation of the possible causes of increased toxicity at higher R.H. revealed that all these insecticides possessed greater fumigant activity at 90%±5% R.H. Diazinon, malathion, and parathion showed no detectable fumigation effect at 55%±5% R.H., but produced mortalities of 100%, 68%, and 55%, respectively, at 90%±5% R.H. with similar temperature conditions prevailing. Heptachlor, heptachlor epoxide, and aldrin residues produced mortalities of 76%, 73%, and 72%, respectively, when assayed at 55%±5% R.H., but when assayed at 90%±5% R.H. the mortalities were 96%, 95%, and 93%, respectively.

It may be postulated that the higher mortality obtained at the higher R.H. is caused largely by increased volatility as shown by the increased fumigant action. This view is also supported by a comparison of the loss in weight of heptachlor epoxide residues held at 90%±5% R.H. and at 55%±5% R.H., under similar temperature conditions. It is concluded that atmospheric humidity greatly affects toxicity of chemicals and for the most efficient use of insecticides they should be applied when the R.H. is high.

A variety of factors determine the residual life of insecticides but perhaps the most important are those of the physical environment. Results reported by other workers indicate the importance of physical factors of the environment in relation to residual life. Sweetman (1945) reported that temperature in the range of 32° to 37° C. with high humidity conditions decreased the residual toxicity of DDT. A similar effect was noted by Burgess & Sweetman (1949). Teotia & Dahm (1950) reported that high temperature and low humidity shortened residue life of a number of insecticides more than low temperature and high humidity. Further studies of these factors were reported by Gaines & Dean (1949), Gaines & Mistric (1952), Mistric & Martin (1956), and Hightower & Gaines (1960).

Sunlight and ultraviolet light also have an adverse effect on insecticide residues (Lindquist et al. 1946, Chisholm et al. 1949, Chisholm & Koblitsky 1947, Fleck 1949, Gunther et al. 1946).

Gaines & Mistric (1951, 1952) reported that the toxicity of parathion and aldrin residues was greatly reduced by simulated rain, dieldrin and toxaphene being less affected. Mistric & Martin (1956) showed a similar effect with parathion and in addition a 4 to 5 mile per hour wind also had an adverse effect on residual toxicity. Dustan et al. (1947) reported that exposure of DDT to air currents for periods up to 6 days had little influence in reducing its effectiveness, but it greatly reduced the effectiveness of those insecticides having pronounced fumigant action.

Busvine (1957) while reviewing the action of humidity stated, "the information on the effects of atmospheric humidity on insecticidal action is fragmentary, conflicting, and usually unexplained. In considering these data it is often impossible to decide whether the effect is upon the insect, the insecticide, or both." The authors are inclined to agree with Busvine but feel that the results of their experiments reported herein more definitely determine the part played by humidity in residual toxicity of insecticides.

PROCEDURE.—The test insect used was the red flour beetle, *Tribolium castaneum* (Herbst). The rearing was essentially the same as described by Peterson (1955) except that new culture jars were started at weekly intervals. The temperature of the rearing room was maintained at 80°±2° F. Three- to four-week-old adults were used in all experiments.

The insecticide treated surfaces consisted of Whatman No. 1 filter papers (9-cm. diameter) uniformly treated with 0.75 ml. of the desired insecticidal concentration. The treated papers were kept in drying racks in the laboratory for 24 hours prior to inauguration of tests.

The test insects were exposed to the toxic residues by placing the treated papers in petri dish halves. Then a glass ring, 5.5 cm. in diameter and 2.5 cm. in height, was placed over the center of each treated paper and the desired number of adult beetles liberated inside. A screen cover was placed over the ring and held in place by rubber bands.

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[2] Graduate student and Fellow of the Rockefeller Foundation, New York. Present address: Government Agricultural College, Ludhiana, Punjab, India.

[3] Professor of Entomology.

[4] Technical Assistant.

Adult beetles were exposed to the treated surfaces for 3 hours. Following exposure the beetles were transferred to clean petri dishes and held in the laboratory for 48 hours after which mortality counts were made. The following criterion of death was established. The beetles were placed in the center of the dish on a piece of paper toweling about 6 cm. in diameter. All the beetles that crawled away from the paper were considered alive; those which either did not move at all, or if they were moving but lying on their backs, when placed on their legs tried to walk but fell over on their backs again, were considered as dead or moribund.

Two temperatures (90° F. and 110° F.) were selected for experimentation purposes and these were maintained by a constant-temperature cabinet showing a variation of ±1° F.

The humidity chambers consisted of ordinary glass desiccators, 10 inches in diameter, with lids fitted with rubber stoppers. A relative humidity of 55% ± 5% was maintained by a saturated solution of calcium nitrate, and a relative humidity of 90% ± 5% was maintained with water. A continuous stream of dry air was conditioned to the respective temperature and humidity and bubbled through each chamber at the rate of 80 ml. per minute. This was to vent any vapors from the insecticide treated papers as rapidly as breakdown occurred.

The insecticide-treated papers were kept in the same racks previously used for drying and the racks were stored inside the humidity chambers which in turn were placed inside the controlled-temperature cabinets.

Emulsifiable concentrates of Diazinon® (*O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate), parathion, malathion, heptachlor, and aldrin, and an acetone solution of heptachlor epoxide were tested.[5]

Preliminary experiments were carried out to determine concentrations that would give comparable mortalities of 90% or more. The initial toxicity of the residue deposits was assayed by using five replicates of 20 insects each. These results represent the percentage mortality of the test insects 1 day after the treatment of the papers.

RESULTS.—The toxicity of Diazinon residues stored at 90° F. and 55% ± 5% R.H. at various intervals of time is shown in figure 1. The results repeatedly indicate the recovery of toxicity following apparent breakdown. On

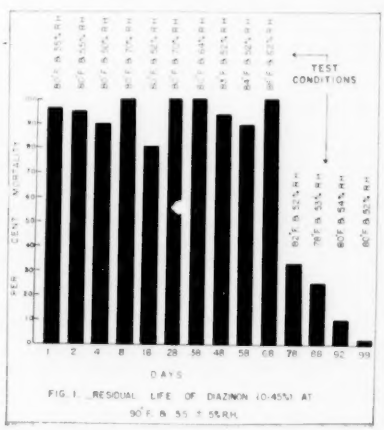

FIG. 1.—RESIDUAL LIFE OF DIAZINON (0.45%) AT 90° F. & 55 ± 5% R.H.

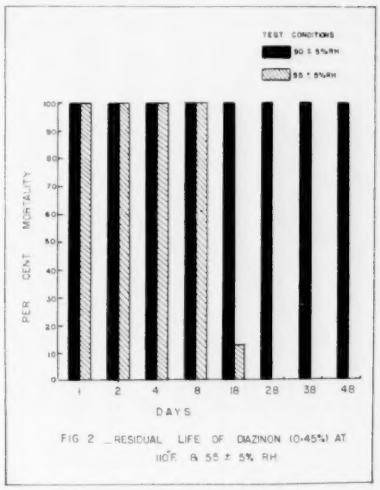

FIG. 2.—RESIDUAL LIFE OF DIAZINON (0.45%) AT 110° F. & 55 ± 5% RH

the 18th day the toxicity was 81% but unexpectedly on the 28th day the residues gave 100% mortality. This variation continued until the 68th day when real breakdown began. Variations likewise occurred in tests with parathion and heptachlor which were conducted simultaneously with Diazinon (figs. 3 and 5).

These evaluations of toxicity were conducted under conditions prevailing in the laboratory, i.e., a temperature of 80° ± 2° F. with considerable variation in R.H. (relative humidity). The rise in toxicity of Diazinon when it once showed apparent breakdown could not be explained by the changes in temperature. These data (fig. 1) suggest a positive relationship between toxicity and humidity at the time of bioassaying. This demanded a change in testing technique and the remaining experiments were carried out in chambers previously described, under the controlled R.H.'s of 90% ± 5% and 55% ± 5%.

For these tests, 10 filter papers were treated with the desired concentration of toxicant and stored at the respective temperature and humidity. At each test the papers were randomly divided into two sets of five, with 20 beetles confined to each paper. One of these sets was placed in the high humidity chamber and the other in the low humidity chamber for the exposure period of 3 hours. Then the test insects were transferred to clean petri dishes and held at room temperature and humidity, and the treated papers were returned to their respective temperature and humidity chambers.

The results are shown graphically in figures 2 to 12. A comparison of the two respective figures for each compound reveals that the higher temperature shortens the life of the residue, and the R.H. at the time of exposure of the insects plays a significant role in toxicity. The toxicity of Diazinon residue was zero 28 days after treatment when assayed at 55% ± 5% R.H. but was 100% when assayed at 90% ± 5% R.H. (see fig. 2) on the 48th day when testing was terminated. This shows that the treated papers still contained toxic residues on the 28th day but

[5] Supplied through the courtesy of the American Cyanamid Co., the Geigy Chemical Corporation, the Shell Chemical Corporation, and the Velsicol Chemical Corporation.

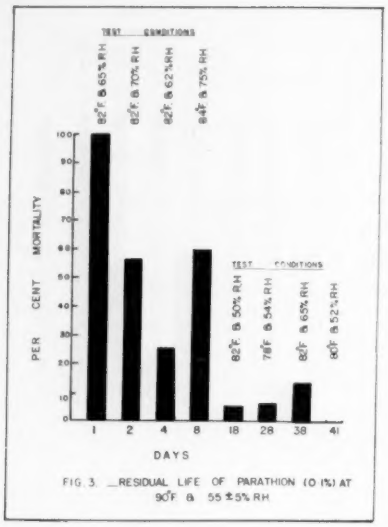

FIG. 3.—RESIDUAL LIFE OF PARATHION (0.1%) AT 90° F. & 55 ± 5% RH.

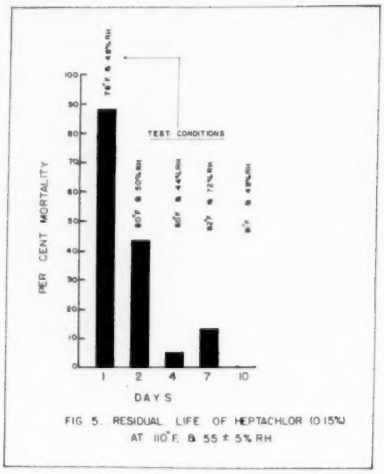

FIG. 5. RESIDUAL LIFE OF HEPTACHLOR (0.15%) AT 110° F. & 55 ± 5% RH.

the toxicity was not evident at 55% ± 5% R.H., whereas the 90% ± 5% R.H. somehow activated the residues to be toxic to the insects or made the insects susceptible to the chemical residue. This same relationship was found to hold for the other insecticides (see figs. 3 to 12).

During these tests it was observed that almost all the insects died within a 3-hour exposure period under high humidity conditions. This suggested the possibility of fumigation effect. An experiment was therefore designed to determine if volatility of these compounds varied with the humidity.

Filter papers were treated and tested as before with the same concentrations of the insecticides, and their contact and fumigant toxicities determined by bioassay at both 55% ± 5% and 90% ± 5% R.H. at a room temperature of 80° ± 2° F. Sixteen papers were treated with each chemical and they were divided into four groups of four each. Twenty-five insects were confined for 3 hours on each paper so that the resulting mortality was based on 100 insects for each group. For checking fumigant effect (no contact) the insects were confined on nylon organdy above the toxic residue. The results are given in table 1.

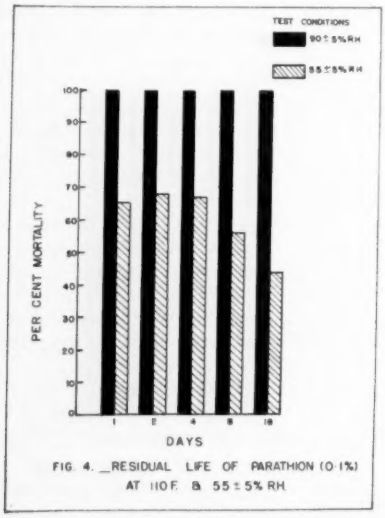

FIG. 4.—RESIDUAL LIFE OF PARATHION (0.1%) AT 110° F. & 55 ± 5% RH.

From these data it is evident that atmospheric humidity plays an important part in increased toxicity of insecticides. It is also evident that volatility of the insecticides increases at the higher humidity as shown by increased fumigant action for all chemicals, but especially for Diazinon, malathion, and parathion. These compounds showed no detectable fumigant effect at 55% ± 5% R.H., whereas at 90% ± 5% R.H. they showed significant fumigation effect.

An additional test was conducted for each insecticide with the insects in contact with the treated papers at 55% ± 5% R.H. for 3 hours, after which the treated papers were transferred to 90% ± 5% R.H. with the insects suspended above the residues. The initial mortalities were the same as given in table 1 for contact at 55% ± 5% R.H., whereas at 90% ± 5% R.H. the mortality for all compounds was 100%. This result indicates that the insects at 55% ± 5% R.H. had picked up sublethal doses at that humidity which were made manifest when placed

Table 1.—The effect of humidity at the time of bio-assay on the toxicity of insecticides—contact and fumigant action.

Insecticide	Concentration (%)	Percentage Mortality—Assayed at Temperature: 80 ± 2° F. 55% ± 5% R.H. Contact	55% ± 5% R.H. No Contact	90% ± 5% R.H. Contact	90% ± 5% R.H. No Contact
Malathion	2.50	90	0	100	68
Parathion	0.10	65	0	100	55
Diazinon[a]	0.45	0	0	100	100
Heptachlor	0.15	100	76	100	96
Heptachlor epoxide	0.15	100	73	100	95
Aldrin	0.10	100	72	100	93

[a] In this instance the same treated papers for the tests reported on in figure 2 were used. They were 48 days old when testing with them was terminated.

at the higher humidity even though the insects were no longer in contact with the residue.

To clarify the problem further another experiment was conducted to compare the loss in weight of an insecticide deposit held at 55% ± 5% R.H. with one held at 90% ± 5% R.H. at a temperature of 80° ± 2° F. Air was passed through the humidity chambers at the rate of 40 ml. per minute to vent possible vapors.

A measured quantity of an acetone solution of heptachlor epoxide was placed in each of seven aluminum

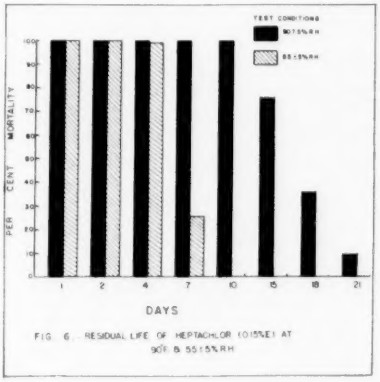

FIG. 6.—RESIDUAL LIFE OF HEPTACHLOR (0.15%E) AT 90°F. & 55±5% R.H.

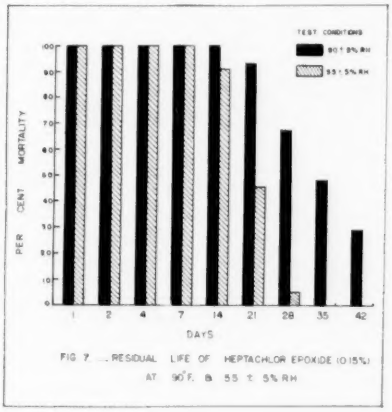

FIG. 7.—RESIDUAL LIFE OF HEPTACHLOR EPOXIDE (0.15%) AT 90°F. & 55 ± 5% R.H.

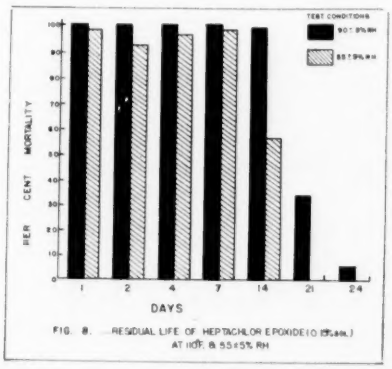

FIG. 8.—RESIDUAL LIFE OF HEPTACHLOR EPOXIDE (0.15%E) AT 110°F. & 55±5% R.H.

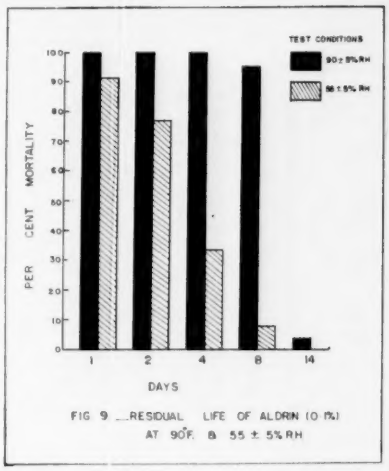

FIG. 9.—RESIDUAL LIFE OF ALDRIN (0.1%) AT 90°F. & 55 ± 5% R.H.

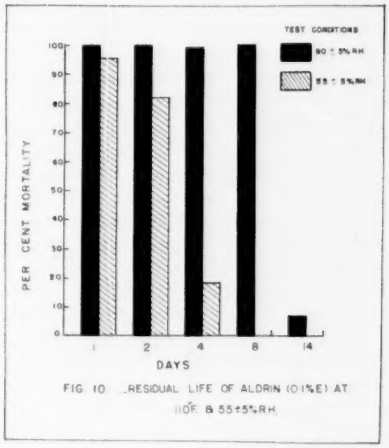

FIG. 10.—RESIDUAL LIFE OF ALDRIN (0.1%E) AT 110°F. & 55±5% R.H.

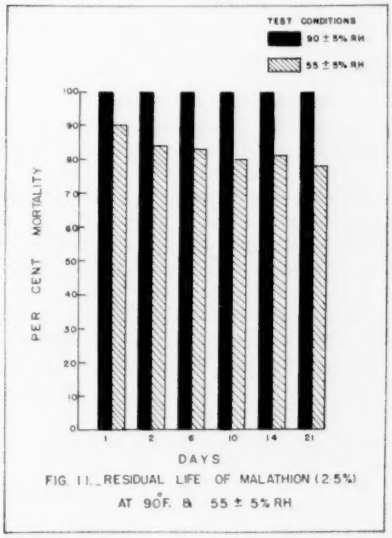

FIG. 11.—RESIDUAL LIFE OF MALATHION (2.5%) AT 90°F. & 55 ± 5% R.H.

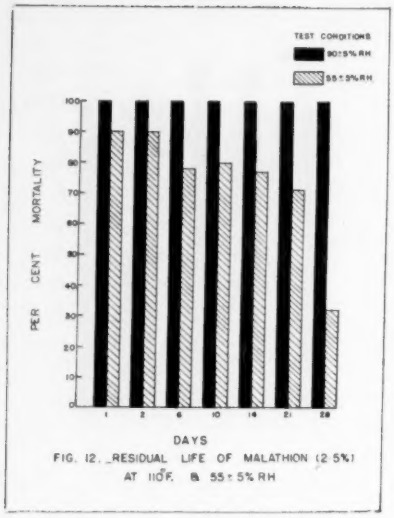

FIG. 12.—RESIDUAL LIFE OF MALATHION (2.5%) AT 110°F. & 55±5% RH

planchets and allowed to dry for 24 hours. Check planchets receiving only acetone were also prepared. All planchets were carefully weighed under standard conditions before and after 10 days exposure of the residues to the test conditions. It was found that heptachlor epoxide residue definitely lost weight more rapidly at the higher humidity. Because of a possible error in procedure this experiment was repeated using larger amounts of heptachlor epoxide on frosted glass plates at a temperature of 94°±2° F. and at relative humidities of 7%±5% and 70%±5%. The air flow through the chambers was 10 cu. ft. per hour. Careful weighings were made under standard conditions before and after 12 days' exposure of the residues to the test conditions. There were five replicates for each R.H. and five replicates for the checks. The results are presented in table 2.

From these results it is evident that the loss of residue is more than two times greater at the higher humidity, corroborating the data in table 1 which show that increased toxicity at the higher humidity is largely caused by a rapid change of the toxicant into a vapor phase in amounts high enough to produce a fumigation effect.

These results certainly help to explain the discrepancies in data obtained by other workers and stress the need for rigid control of test conditions for all who evaluate the toxicities of chemicals. The need for the establishment of standard test conditions is emphasized. The results from these experiments also indicate that an insecticide, if used in the field (under similar conditions except for variations in humidity) at a very high humidity will give higher immediate kill but will be lost more rapidly than under dry conditions. In addition the dosage required in humid areas need not be so high as in arid areas in order to give the same degree of control, if other factors of the environment remain the same. For more efficient use of the insecticides tested they should be applied when the humidity is high.

Table 2.—Effect of humidity on the volatility of heptachlor epoxide at 94°±2° F.

	7%±5% R.H.		70%±5% R.H.	
	Initial Weight (mg.)	Loss in Weight (mg.)	Initial Weight (mg.)	Loss in Weight (mg.)
	128.4	2.0	139.3	5.7
	125.9	2.8	123.6	4.6
	124.0	4.3	125.1	5.6
	124.4	3.1	128.7	5.4
	131.3	3.0	133.8	9.0
Total	634.0	15.2	650.5	30.3
Check gain		1.2[a]		.1[a]
Corrected value		14.0		30.2

[a] Check gain was probably caused by impurities in the air flow or by dust deposited during weighing.

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Resistance of Squash Varieties to the Pickleworm and the Value of Resistance to Insecticidal Control[1]

CHARLES H. BRETT, C. L. MCCOMBS, and D. M. DAUGHERTY, *North Carolina State College, Raleigh*

ABSTRACT

Sixteen varieties of squash were tested for resistance to pickleworm, *Diaphania nitidalis* (Stoll). Differences were very distinct between the more resistant and the more susceptible varieties. The more resistant varieties were Butternut 23, Summer Crookneck, Early Prolific Straightneck, and Early Yellow Summer Crookneck. The more susceptible varieties were Cozini, Black Zucchini, Caserta, Zucchini, Short Cocozelle, and Benning Green Tint Scallop. Tests with insecticides showed that better control could be obtained on resistant than on susceptible varieties. The peel and interior fruit portions of seven varieties were analyzed for amino acids and carbohydrate materials. There was no clear evidence of correlation between chemical composition and resistance. Galacturonic acid occurred only in the most resistant variety, Butternut 23, suggesting that further study should be made regarding the influence of this compound.

The pickleworm, *Diaphania nitidalis* (Stoll), is one of the most destructive insects attacking late summer-grown squash in North Carolina. It also infests other cucurbits, but squash appears to be its favorite food. Quaintance (1901) recommended the use of squash as a trap crop to protect cantaloupes and cucumbers. He found that squash flowers were heavily infested while cucumber flowers were still clean. Britton (1935) reported that in Connecticut early crops of cucumbers and cantaloupes nearly always escaped injury and that later crops could be protected by successive plantings of squash every 2 weeks (4 rows to the acre) to furnish blossoms upon which the moths might lay their eggs. Larvae could then be destroyed by removing the blossoms. Fulton (1937) in North Carolina observed that cantaloupes grown beside squashes did not become infested until early in August and never to the extent that the squashes were infested. Watermelons were seldom attacked. Bodenstein (1944) found that cucumbers, growing in a plot adjoining squash test plots, were practically free from pickleworm injury.

Although these observations did not include a comparison of varieties, they indicate that in general squash is one of the cucurbits most susceptible to the pickleworm. The nature of resistance was touched upon briefly by some observers, but no attempt was made to establish physiological or morphological associations.

Fulton (1947) observed that young tender fruits of squash, pumpkins, and gourds were more susceptible than the older fruits. He considered that older fruits became largely immune because of hardness of the rind and that this characteristic varied in different varieties. In some varieties of winter squash he found the fleshy fruit stem was usually the only part attacked. He ranked acorn squash as intermediate between resistant winter squash and susceptible summer squash. Howe (1949), in a study of factors affecting the resistance of certain cucurbits to the squash borer, suggested that *Cucurbita moschata* (5 varieties of Butternut) was more resistant than *C. pepo* (Early Summer Crookneck and White Bush Scallop) and *C. maxima* (Blue Hubbard, Golden Delicious, and Boston Marrow) because it was the more primitive species and that it may be more resistant to all insects owing to its longer exposure time. Varieties of *C. pepo* were somewhat resistant because of an abundance of succulent tissue in large fast-growing stems.

The causes of resistance are seldom known. Painter (1951) says there are many possible causes of resistance and even a high correlation coefficient is only suggestive and by itself does not constitute proof. He considers that a knowledge of the cause of resistance is desirable, but it may or may not be of use in breeding operations. Bailey (1941) expressed the opinion that it was not necessary to know the cause of resistance and stated that "vegetable crops probably offer the greatest opportunity today for original work in the field of breeding for insect resistance. . . ."

The multiplicity of factors involved in resistance necessitates a broad study of the plant-insect interrelationship. Painter (1941) concluded that resistance could be defined under the terms tolerance, preference, and antibiosis and has discussed these in detail (Painter 1943, 1951).

Importance of the pickleworm as a pest of squash and the lack of information regarding resistance in squash varieties to this insect prompted the present study. It was hoped that some associated factors might be found which would suggest something of the nature of resistance involved and for this reason fruits were analyzed for their content of carbohydrates and amino acids in addition to scoring them for insect damage.

METHODS.—Sixteen commercial varieties of squash were selected for this study. Seed was supplied by Associated Seed Growers, Inc. Pickleworms generally make their appearance during the first part of July in the area of Faison, North Carolina. Planting in 1959 was done August 3 in order that fruits would begin development during a period of high population. Hills were 6 feet apart and 10 hills were planted per plot. Each hill was thinned to two plants. Each variety was planted in three randomized replicates. Fruits were picked and scored September 19, 24, October 1, 8, 15, 22, 29, and November 5. Scoring included counting the fruits, determining, whenever possible, the number of fruits that were destroyed, counting the number of entry holes per fruit, the number of incomplete entries (pits), and the number of clean fruits.

In 1960, varieties were selected on the basis of the performance they had shown in 1959. Butternut and Summer Crookneck were ranked as the most resistant varieties. Benning Green Tint Scallop, Caserta, and Black Zucchini were ranked as the most susceptible varieties. Except for Early Prolific Straightneck, Black Beauty, and Black Zucchini, these were all compared under treatments with insecticide dusts containing 1% lindane, 1¼% Sevin® (1-naphthyl *N*-methylcarbamate) and in the case

[1] Contribution from the Entomology Department and the Horticulture Department, North Carolina Agricultural Experiment Station, Raleigh. Published with the approval of the Director of Research as Paper No. 1321 of the Journal Series. Accepted for publication June 5, 1961.

Table 1.—Resistance of squash varieties to pickleworm. 1959.

Variety	Number of Entries per Infested Fruit	Infested Fruit (%)	Infested Fruit (%) Transformed to Angles[a]
Butternut 23	1.4	19	25.91
Summer Crookneck	1.7	27	31.55
Early Prolific Straightneck	2.2	30	33.38
Early Yellow Summer Crookneck	1.9	32	33.75
White Bush Scallop	6.0	45	42.03
Black Beauty	2.4	60	54.49
U Conn	3.1	62	52.00
Marine Black Zucchini	3.0	66	57.17
Cozella Hybrid	2.9	70	57.12
Long Cocozelle	4.8	70	58.43
Benning Green Tint Scallop	4.1	71	56.83
Short Cocozelle	2.4	77	63.49
Zucchini	2.1	80	66.39
Caserta	5.1	82	66.79
Black Zucchini	3.4	85	65.69
Cozini	11.4	100	90.00

[a] L.S. D at 5% level=11.5; at 1% level=15.52.

of Benning Green Tint Scallop 4% Dibrom® (1,2-dibromo-2,2-dichloroethyl dimethyl phosphate) was also included. Dusts were applied at the rate of 30 pounds per acre. Each plot consisted of 8 hills 8 feet apart with 2 plants per hill. Seeds were planted August 3, and dusts were applied at the rate of 30 pounds per acre September 9, 23, and October 10. Infestations were scored in the manner described for 1959 and scoring was done September 15, 23, 30, October 8, 14, 21, and 28.

Fruits of the varieties grown in 1959 were analyzed for amino acid composition utilizing longitudinal slices from each of six fruits per variety. Fruits harvested in 1960 for analysis were separated into peel and interior portions to enhance the detection of compounds which might possibly be present only in the peel portion. Both portions were analyzed for the carbohydrates and amino acids present in the alcoholic extracts. The plant extracts were prepared as described by Plaisted (1958). The residues from the lyophilized resin-treated extracts were dissolved in 1 ml. of 10% isopropyl alcohol and various amounts spotted on Whatman No. 1 paper for chromatographic analysis. The amino acids were separated two-dimensionally employing the solvent system reported by Benson et al. (1950). Detection was accomplished by dipping the developed chromatograms in 0.5% ninhydrin in acetone. Since the immediate interest in this phase of the study was qualitative, evaluation was performed only by visual comparison of the size and intensity of color of spots with those on the chromatograms of three levels of concentration of amino acid standards. Identification was ascertained by co-chromatography of known amino acids.

The sugars were separated on one-dimension strips using ethyl acetate: acetic acid: water (10:5:2) and detected by dipping the developed chromatograms into 2-aminobiphenyl followed by heating to produce the color (Block et al. 1958).

RESULTS AND DISCUSSION.—In this study, resistance to pickleworm was evaluated as per cent infestation, since from the standpoint of fruit quality the presence of a single insect larva or external evidence of larval damage essentially destroys the market value. Relative resistance in 1959 of the 16 varieties of squash is shown by their position in table 1. It will be noted that these percentages are based on the total number of fruits picked each week for a period of 8 weeks. Infestations varied during this period with the result that the position of the varieties in any given week would not hold exactly the same relationships that they might during some other week. Because of the complex interrelationships which exist between plant and insect, the relative attractiveness of different varieties may vary under different conditions. Figure 1 shows the variation in per cent infestation which occurred week by week for three of the most resistant varieties (Butternut 23, Summer Crookneck, and Early Prolific Straightneck) and three of the most susceptible varieties (Black Zucchini, Cozini, and Caserta). The period from September 19 to October 8 was hot and dry which might have accounted for the downward trend in the percentage of infested fruit. During the remainder of the season, there was a total of 5½ inches of rainfall and low temperatures prevailed. Fluctuations in percentage of infested fruit during this time apparently were associated with hatching and the number of fruits available to the larvae. A resistance classification of the botanical groups of squash shows *Cucurbita moschata*, of which Butternut is a representative, to be the most resistant, followed by the Summer Crookneck group of *C. pepo*. Other groups represented in this experiment were in an unordered relationship.

In some varieties the plant stems, flowers, and young fruits were more severely attacked by pickleworms than in others. Many very young fruits were destroyed in Benning Green Tint Scallop. Much injury was also observed in the stems and flowers of this variety. Considerable injury in the stems was also evident in Cozini. This variety was a poor producer and more than 50% of the fruits were destroyed in their early stages of development.

Cozini, Black Beauty, and Caserta were more exten-

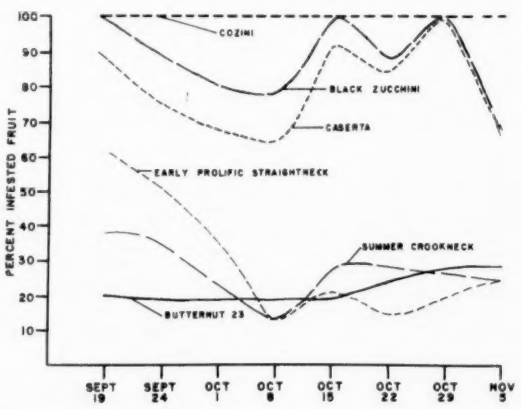

FIG. 1.—Variation over an 8-week period, in the per cent infestation by pickleworm of three of the most resistant and three of the most susceptible varieties of squash.

sively damaged by striped cucumber beetles, *Acalymma vittata* (Fabricius), feeding on the leaves, than any of the other varieties. Butternut 23, Summer Crookneck, Early Prolific Straightneck, Early Yellow Summer Crookneck, and White Bush Scallop were good producers and very little damage occurred in the young fruits. However, during the middle of September a population of adult squash bugs, *Anasa tristis* (De Geer), concentrated on the Butternut 23 plots. Before these bugs could start a new generation they were destroyed by a tachina fly, *Trichopoda pennipes* Fabr.

Results from tests made in 1960 are shown in table 2. These data are based on weekly harvests taken over a 7-week period. Butternut 23 was so lightly infested that it approached immunity. No evidence of pickleworm injury in this variety was present until October 28, and this injury was so light that differences between insecticide-treated plots and untreated plots were not significant. Such injury as did occur was probably caused by transient larvae from adjacent or nearby plots which carried higher populations. Black Zucchini and Caserta were apparently more susceptible to pickleworm than Benning Green Tint Scallop. At the 5% level of significance they were more susceptible than Summer Crookneck or Butternut 23. A very high percentage of the young fruits on Caserta were destroyed by pickleworm. Benning Green Tint Scallop was apparently more susceptible than Summer Crookneck and was significantly more susceptible than Butternut 23 (see figs. 2 and 3). Summer Crookneck was also significantly more susceptible than Butternut 23.

The difference in resistance or susceptibility of these varieties was reflected in the control (based on per cent infested fruit) of pickleworm with insecticides. In all tests lindane appeared to give better control than Sevin although the difference was not significant when comparisons were made on the same variety. However, control with Sevin on Caserta, the most susceptible variety treated, was significantly less effective at the 5% level than lindane on all varieties other than Caserta and was significantly less effective than Sevin on Butternut. Lindane was significantly less effective on Caserta than on Butternut and was nearly significantly less effective on Summer Crookneck.

Table 2.—Effect of resistance in squash varieties to the pickleworm, on control by insecticides. 1960.

Variety	Treatment[a]	Number of Entries per Infested Fruit	Infested Fruit (%)	Infested Fruit (%) Transformed to Angles[b]
Butternut 23	Lindane 1%	1.0	2	3.27
	Sevin 1¼%	1.0	2	7.77
	Untreated	1.0	3	11.37
Summer Crookneck	Lindane 1%	1.0	2	6.27
	Sevin 1¼%	1.4	10	18.03
	Untreated	2.0	49	40.43
Early Prolific Straightneck	Untreated	3.9	46	44.13
Black Beauty	Untreated	3.5	56	48.97
Benning Green Tint Scallop	Lindane 1%	1.0	3	8.60
	Sevin 1¼%	1.4	13	15.80
	Dibrom 4%	2.1	35	—
	Untreated	3.5	62	50.17
Caserta	Lindane 1%	1.0	10	17.37
	Sevin 1¼%	1.4	20	20.40
	Untreated	3.1	72	58.63
Black Zucchini	Untreated	3.6	72	58.50

[a] Dusted at the rate of 30 pounds per acre September 9, 23, and October 10.
[b] Significance at the 5% level = 14.18, at the 1% level = 19.27.

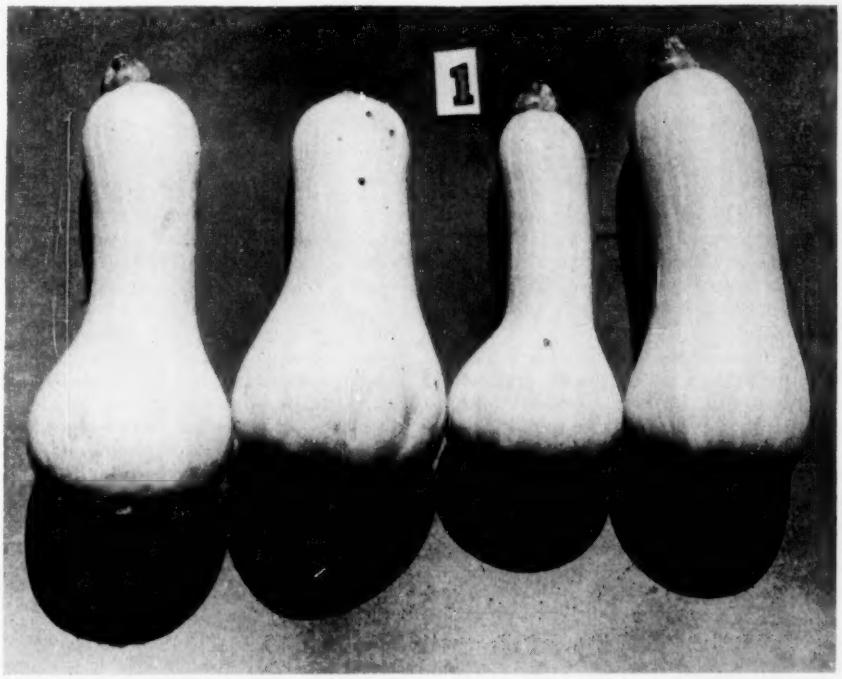

FIG. 2.—Butternut 23 squash. Resistance to pickleworm is shown by lack of injury to the fruit.

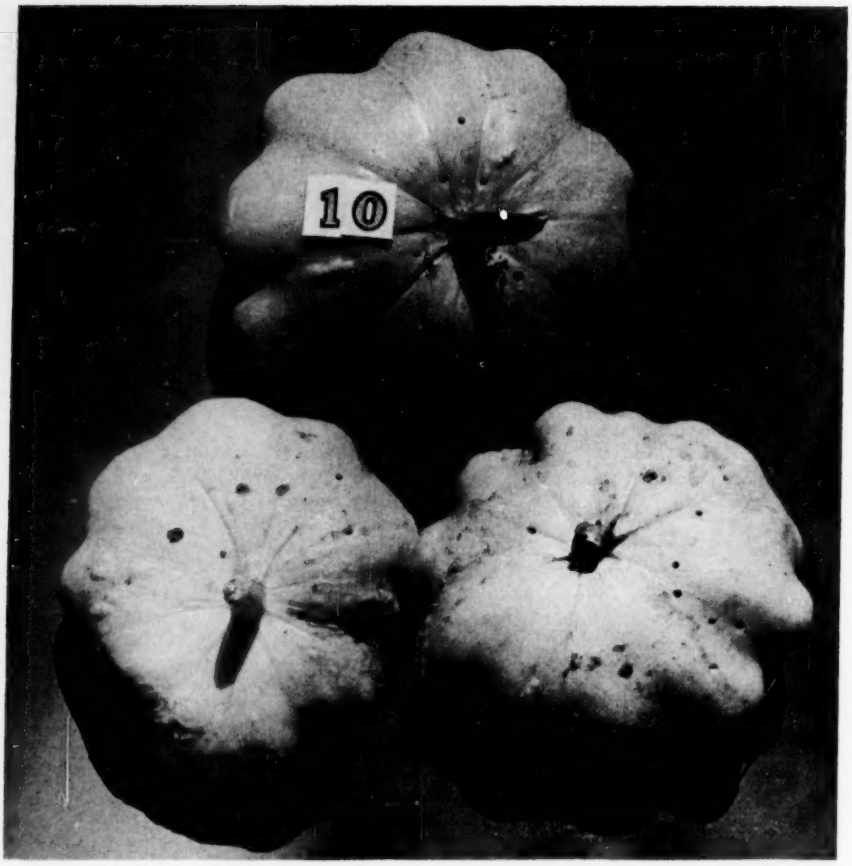

FIG. 3.—Benning Green Tint Scallop squash. Susceptibility to pickleworm is shown by extensive damage to the fruit.

Sevin gave control on Benning Green Tint Scallop that was significantly less effective than lindane on Butternut or Summer Crookneck. Lindane was apparently less effective in its control on Benning Green Tint Scallop than on Butternut or Summer Crookneck but this difference was not significant.

Control with Sevin on Summer Crookneck was significantly less effective than with lindane on Butternut. Lindane was apparently less effective on Summer Crookneck than on Butternut but the difference did not reach significance.

These results show that plant resistance to the insect gave the double advantage of (1) a lower level of infestation and injury when untreated and (2) an even lower level when treated with insecticides.

Early Prolific Straightneck and Black Beauty were not treated with insecticides. Their position in table 2 shows these varieties, as well as the others, hold the same relative position as in table 1 and thus substantiate the 1959 test. Striped cucumber beetle damage in 1960, as in 1959, was most extensive on Black Beauty and Caserta.

Benning Green Tint Scallop was an excellent variety for comparing the effectiveness of different insecticides. It attracted pickleworms sufficiently to build up a high infestation and was a prolific producer. Furthermore, the injury to fruits was easily seen and this factor facilitated scoring. The selection of such a variety for field testing insecticides is one of the bonuses from such a study as this. Four per cent Dibrom was also included in the tests on Benning Green Tint Scallop. This material was less effective than either Sevin or lindane. Although Dibrom has given very good control in other pickleworm tests in North Carolina, its short residual effect probably accounted for its comparative ineffectiveness in this experiment.

A total of 23 amino acids and three other ninhydrin-positive spots were observed on the developed chromatograms of the plant extracts of the 15 varieties included in 1959 sampling. The amino acids present in the greatest amounts were serine, alpha-alanine, aspartic acid and its amide, asparagine. Fifteen of the 21 amino acids were present in the extracts of all of the varieties. Proline was detected in only four varieties and leucine in two varieties. No correlation was observed between resistance or sus-

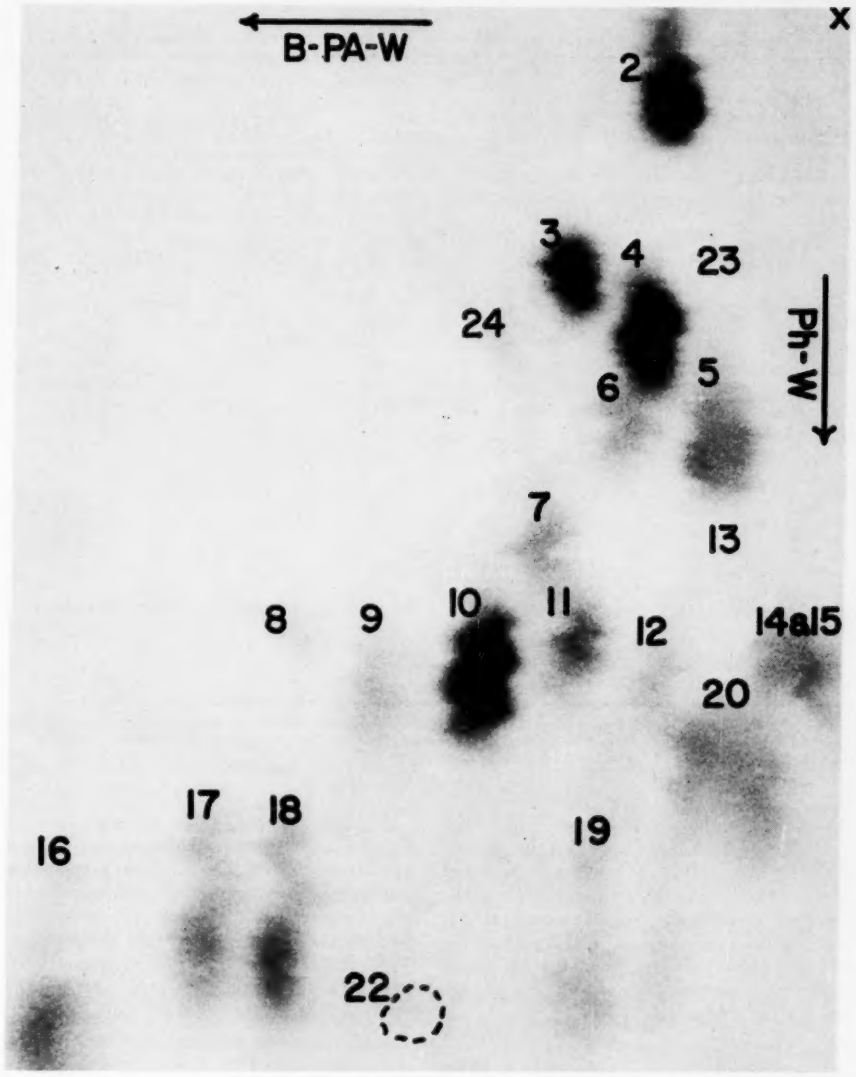

FIG. 4.—Chromatogram of ninhydrin-positive compounds in peel extract of Butternut 23 squash fruit. Spots are somewhat larger and darker for photographic purposes than would appear on typical chromatogram. 1 Cysteine, 2 Aspartic acid, 3 Glutamic acid, 4 Sevine, 5 Asparagine, 6 Glycine, 7 Threonine, 8 Tyrosine, 9 B-alanine, 10 a-alanine, 11 Unknown #1, 12 Glutamine, 13 Ornithine, 14–15 Histidine and Arginine, 16 Leucine, 17 Valine, 18 9-Aminobutyric acid, 19 Methionine, 20 Unknown #2, 21 Phenylalanine, 22 Proline, 23 Taurine, 24 Unknown #3.

ceptibility to pickleworm infestation, and the presence or absence of a particular amino acid or a ninhydrin-positive spot.

The results of the analyses of the peel and interior portions of the fruits of each of seven varieties sampled in 1960 are presented in tables 3 and 4.

For five of the seven varieties there are marked differences between the peel and interior portions with respect to their amino acid complement. In the variety Butternut 23, which is the least susceptible to pickleworm infestation, the greatest number and amounts of amino acids were detected in the peel. Summer Crookneck was similar in this respect but to a lesser degree. Conversely, fruits of the varieties Early Prolific Straightneck and Caserta contained more amino acids in the internal than in the peel portion. The most susceptible variety, Black Zucchini, contained approximately the same kinds of amino acids in both portions of the fruit but the greatest amounts were also present in the internal part. Differences were minimal in the varieties Benning Green Tint Scallop

Table 3.—The amino acids and amides present in the peel and interior (Int.) portions of squash fruit.[a]

Amino Acids or Amides	Varieties													
	Butternut		Summer Crookneck		Early Prolific Straightneck		Black Beauty		Benning Green Tint Scallop		Caserta		Black Zucchini	
	Peel	Int.[b]	Peel	Int.	Peel[b]	Int.	Peel	Int.	Peel	Int.	Peel[b]	Int.	Peel	Int.
a-Alanine	++	ft	++	+	ft	+	++	+	+		ft	+	ft	++
b-Alanine	+		+	+		+	+	+	+	+		+		+
Asparagine	++		++	+		++	+++	+	++	+++		++	ft	+++
Aspartic	+		+	ft	ft	+	+	+	+	+		+	ft	+
a-amino butyric	+		+	+		+	+	+	+	+		+	+	++
g-amino butyric	++	+	++	+	ft	+	++	+	+	+	+	+	+	+
Citrulline							ft	ft		ft				ft
Cysteine	+		+				+	+	+	+		ft	ft	ft
Ethionine	+	++	+		++	+	+	+	+	+	++	+	++	+
Glutamic	+		+	ft	ft	+	+	+	+	+	ft	+	ft	+
Glutamine	+		+	ft		+	++	++	+	++		+	ft	++
Glycine	+	+	+	ft	ft	+	+	+	+	+	ft	+	ft	++
Leucine	+		+	+	ft	+	++	+	+	++	+	+	ft	+
Methionine	+		+			+	+	+	+	+		+		+
Ornithine	+		+			+	+	+	ft	+		ft	ft	ft
Phenylalanine	+		+	ft		ft	+	+	ft	ft	ft	ft		+
Pipecolic								ft				+		+
Proline	+		+	+	+	+	+					+		+
Serine	++		+	ft	ft	+	++	+	+	+++	ft	+	ft	++
Taurine	ft		ft			+		+	+	+				ft
Threonine	+	ft	+	ft		+	+	+	+	+		+	ft	+
Tyrosine	+	+	+	+		+		+	+		ft	+	+	+
Valine	+	ft	+	ft	ft	+	+	+	+	+		+	ft	+
Unknowns 1	+		+	ft		++	+	+	+	+		+	ft	ft
2	++	++	+	+	+++	+	++	ft	+	+	+++	+		+
3	+	ft	ft			+	+	+	ft	+		ft	+++	ft

[a] Each + symbol is equivalent to approximately 5 micrograms of the known compounds and is representative of the amount in 1 gram of plant material except as noted. Lesser amounts of the compounds present are indicated by "ft" (faint color). No symbol indicates the compound was not detected.
[b] The values indicated are representative of the amount in 2 grams of plant material.

and Black Beauty. If the amino acid complement of the entire fruit is considered, that is the peel plus the interior portion, a similarity among the varieties will be noted except for citrulline and pipecolic acid. Correlation between susceptibility or resistance to pickleworm infestation and the amino acid composition of the peel or the internal portions of these varieties which are known to differ in their degree of susceptibility to pickleworm infestation is not readily evident.

A number of carbohydrate materials were present in the alcoholic extracts although in some cases it was necessary to spot seven times the minimum volume to detect compounds present in small amounts. Unlike the amino acid complements there were few differences between the two portions of the squash fruit. The higher carbohydrate was not identified but it is a compound of low Rf value moving less than 1 inch from the origin during a 48-hour development period. Of most interest was the detection

Table 4.—Extractable carbohydrates present in the peel and interior (Int.) portions of squash fruit.

Carbohydrates	Butternut		Summer Crookneck		Early Prolific Straightneck		Black Beauty		Benning Green Tint Scallop		Caserta		Black Zucchini	
	Peel	Int.	Peel	Int.	Peel	Int.	Peel	Int.	Peel	Int.	Peel	Int.	Peel	Int.
Higher Carbohydrate	+	+	+	+			+	+				+	+	
Arabinose		ft				ft		ft				ft	ft	ft
Cellobiose	+	+	+	+	+	ft	+	ft	+	+	+	+	+	+
Fructose	++	++	++	++	++	++	++	++	++	++	++	++	++	++
Galacturonic acid	ft	+												
Glucose	++	++	++	++	++	++	++	++	++	++	++	++	++	++
					+	+	+	++	++	++	++	+	+	+
Sucrose	+	+	+	+	+	+	+	+	+	+	+	+	+	+
(Relative volumes spotted)[a]	1	1	1	1	2	2	7	2	7	2	7	5	7	7

[a] Volumes necessary for detection of carbohydrates present in minimal amounts. Unit volume was 5 microliters which was equivalent to 0.0125 gram of plant tissue. Symbol of one + approximates 50 micrograms, except for the unknown higher carbohydrate compound. "Ft" indicates amounts less than 50 micrograms. No symbol indicates the compound was not detected.

of galacturonic acid in only the most resistant variety, Butternut. The significance of this finding, if any, will be evaluated in further experiments.

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Certain Biological Effects Produced in the Boll Weevil by Tagging It with P^{32} [1]

M. S. MAYER and J. R. BRAZZEL[2]

ABSTRACT

Boll weevils (*Anthonomus grandis* Boheman) were tagged with radioactive phosphorus by feeding to adults in solutions or rearing them in larval diets to which $H_3P^{32}O_4$ had been added in varying quantities. The rates of loss of radioisotope were higher for those weevils fed P^{32} as adults than for the weevils reared on the radioactive larval diet.

Studies of the effects of P^{32} on fecundity, longevity, lengths of oviposition and pre-oviposition periods disclosed that the weevils reared from radioactive larval diet were more adversely affected than the weevils fed as adults. Females reared from the two highest dosages in the diet failed to lay eggs. Larval mortality increased in proportion to the amount of radioactivity in the diet, and mortality was always greater and began sooner for the weevils reared in radioactive larval diet.

The boll weevil, *Anthonomus grandis* Boheman, has been studied extensively in reference to its biology and certain aspects of its physiology and ecology. However, little progress has been made in determining its dispersal and migratory habits. This lack of information is due primarily to the difficulty of relocating the weevils in the field or in the overwintering sites.

During the past 10 years radioisotopes have been employed successfully to follow the movements of various insects under natural conditions. An ideal radioisotope for studies of this type on the boll weevil would have no adverse effects on the biology or habits of this insect. It should be possible to label the insect with sufficient amounts of radioactivity to insure relocation for periods up to 4 or 5 months. These factors require investigation into the length of time a suitable radioisotope will remain upon or in the insect in quantities sufficient for relocation; and, the possible detrimental effects on the insect.

Babers et al. (1956) reported the first attempt to tag boll weevils with a radioisotope. They were able to tag the weevils by dipping them into a water solution of Co^{60} exhibiting 6.6×10^5 counts per minute per milliliter. Results showed that a wetting agent greatly increased the amount of Co^{60} that adhered to the weevils. The effects of the radioisotope on biological functions of the weevil were not investigated.

Since 1949, many species of insects have been tagged with radioisotopes for studies of their migratory habits: Fredeen et al. (1953); Jenkins & Hassett (1951); Lindquist et al. (1951); Schoof et al. (1952); Schoof & Siverly (1954) and others.

Several studies have been made of the effects of P^{32} on various aspects of the biology and physiology of insects: Arnason et al. (1949); Babers et al. (1956); Grosch & Sullivan (1952 and 1953), and others. Most of the results obtained from these studies vary with the species of insect studied.

The studies reported herein were designed to develop a method for tagging the boll weevil with P^{32}, and to determine if the ionizing radiation had adverse effects on certain biological phenomena.

MATERIALS.—The P^{32} employed in these studies was obtained from the Oak Ridge National Laboratories as 20 millicuries (mc.) of $H_3P^{32}O_4$. The first sample exhibited

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[2] Graduate Assistant and Associate Professor, respectively, Entomology Department.

5.7×10^8 counts per minute per milliliter (c.p.m./ml.). During the course of experimentation, another sample of 20 mc. was obtained which was not assayed.

All counts of radioactivity were made on a Nuclear Measurements Corporation Model PC-3A decimal scaler consisting of 3 scale-of-ten circuits with a resolution time of 2 microseconds. A G-M tube with end-window density of 1.4 mg./cm.^{2} was employed to make all counts. No corrections were made for self absorption for any of the counts.

The weevils to be fed P^{32} were confined in cages made from 1-pint ice-cream cartons vented on one end with 14×18 mesh screen wire. Feeding tubes fabricated from $\frac{1}{4}$ inch diameter glass tubing were used to hold the solution containing the isotope. The tubes were prepared by cutting glass tubing to approximately 2 inches in length and fusing one end shut. Absorbent cotton was rolled into a tight plug and inserted into the open end of the tube. A five-sided screen shield was fitted over the cotton plug in the open end of the tube and taped into place to eliminate contamination of all parts of the weevil except the mouth parts. The completed tube assembly was fitted tightly into holes cut in the side of the cage about one-half inch from the bottom with the cotton-plugged end inside the cage.

METHODS.—Two methods were employed to tag the boll weevil with P^{32}. The first method consisted of feeding solutions of the radioisotope to the adults. The labelled phosphoric acid was diluted in distilled water or a 25% honey and water solution and fed to the weevils. This solution was delivered in 1-milliliter quantities to the feeding tubes by a hypodermic syringe. As the volume of the liquid was reduced by the feeding weevils, it was replaced by adding a similar amount of nonradioactive liquid to each of the tubes in the experiment.

A preliminary experiment was designed to determine the uptake and loss of an oral dose of 225 microcuries of P^{32} per milliliter (μc./ml.) of solution. There were 6 replicates of 10 weevils each, with no segregation of males and females. The radioisotope was dispensed in distilled water.

The five-sided screen shield over the cotton feeding plug was not employed in this experiment and all parts of the weevils became contaminated. In subsequent experiments the screen shield over the feeding tube reduced the amount of radioactivity exhibited by the weevils by eight times.

One-half of the weevils were removed from each of the replicates after the second day of feeding and placed on nonradioactive cotton squares. The declining radioactivity of these weevils was determined for 16 days.

In other experiments employing oral doses of P^{32}, males and females were segregated and counted separately to determine if one sex became more radioactive than the other. Seven males and seven females each were fed 148, 25 and 10 μc./ml. of a honey-water solution of the radioisotope. The exhibited radioactivity of each weevil was recorded as counts per minute (c.p.m.) at 24-hour intervals for 72 hours from the time the solution was first administered to them.

A second method for incorporating the P^{32} into the weevils consisted of rearing the weevils in a larval diet which contained approximately 1.65, 4.96 and 9.91 μc./g. of diet. The ranges of concentration of P^{32} in the diet were 1.45–1.85, 4.35–5.56 and 8.70–11.11 for the low, medium and high average concentration, respectively. This method allowed a measurement of the amount of radioactivity accumulated by the weevils in the larval stage by determination of the level of radioactivity exhibited by the adults immediately upon emergence. The diet employed was the standard diet for rearing boll weevils in the laboratory (Brazzel et al. 1959). Distilled water was furnished the control groups instead of nonradioactive phosphoric acid since such small amounts of phosphorus were added in the treatments that it was unlikely that a biological difference could be measured.

The determination of the amount of radioactivity per gram of diet was obtained by weighing the diet after it had been poured into tared petri dishes. The levels of radioactivity were not absolute throughout the duration of the experiments because of the dehydration of the diet and normal disintegration of the radioisotope.

All counts of the exhibited radioactivity of the weevils were made using the same technique. Each weevil was enclosed in a No. 0 gelatin capsule for counting. This confinement was necessary to prevent radical changes in the geometry of the counting chamber by movements of the weevil. The counts were taken for 1- to 5-minute durations with subsequent return of the weevils to constant temperature conditions.

After the third day of adult feeding on radioactive solutions, or upon adult emergence from the radioactive larval diet, the weevils were paired in shell vials stoppered with cotton plugs. For those weevils emerging from the diet, the sex, weight and initial radioactivity were determined immediately. Fresh cotton squares were provided daily for food and oviposition. The declining radioactivity of all weevils was determined daily for 41 days.

The biological loss of P^{32} expressed as a percentage was determined by dividing the c.p.m., corrected for decay, into the c.p.m. exhibited by the weevils the day they were removed from the radioactive food.

The fecundity of the treated weevils was measured by dissecting the cotton squares furnished the preceding day and recording the number of eggs laid by each female. The radioactivity of all eggs laid for 10 days after oviposition began was determined. These eggs were placed in the center of a planchet on damp filter paper and counted for 5-minute durations.

The F_1 generation of the weevils reared in diet containing 1.65 μc./g. were studied along with the F_1 generation of the weevils fed 10, 25 and 148 μc./ml. orally. These studies were conducted in the same manner as studies of the parent generation and were subjected to identical conditions, except that fresh cotton squares were furnished every 2 days. At no time was the F_1 generation exposed to any radioisotope.

RESULTS.—Results of preliminary experiments, presented in table 1, indicated that boll weevils became highly radioactive if allowed to feed on a water solution containing 225 μc./ml. of P^{32}. An average observed activity of 64,856 c.p.m. during the first 24 hours was recorded. By the fifth day, 15 of these weevils averaged 346,001 c.p.m. corrected for decay.

Fifteen weevils removed from the radioactive food after the second day of feeding and placed on nonradio-

Table 1.—Rate of uptake and loss of P^{32} fed at a rate of 225 μc./ml. of distilled water by media-reared boll weevils starved 48 hours prior to treatment.

Days	Number Weevils Counted	C.p.m./ Weevil[a] Observed	Number Weevils Counted	C.p.m./ Weevil[b] Observed	Per cent Loss Biological
1	18	64,856			
2	18	269,009			
3	18	263,057	18	116,013	56.84
4	18	253,721	18	61,318	80.34
5	18	280,269	18	46,629	84.15
13			14	7,359	95.99
16			14	4,179	97.38

[a] Fed P^{32} continuously.
[b] Removed from P^{32} on second day.

Table 2.—Rate of uptake of P^{32} fed at rates of 10, 25 and 148 μc./ml. of a 25% honey-water solution by media-reared boll weevils starved 48 hours prior to treatment.

Dose (μc/ ml.)	Days of Feeding	Number Weevils Counted	Males Observed (c.p.m.)	Number Weevils Counted	Females Observed (c.p.m.)
10	1	7	1,335	7	1,446
	2	7	1,204	7	1,888
	3	6	1,689	6	2,075
25	1	7	3,659	7	2,537
	2	7	6,058	7	7,321
	3	5	5,054	5	5,087
148	1	7	16,437	7	9,690
	2	7	19,923	7	21,968
	3	4	12,388	7	24,667

active cotton squares lost 57% of the radioactivity during the first 24 hours. After 16 days these weevils had lost 97% of the initial level of activity, however, they still exhibited an average of 4,179 c.p.m. Other preliminary experiments indicated that the amount of radioactivity exhibited was proportional to the radioactivity of the food solution.

The next series of experiments was conducted to determine the rate of uptake and loss of P^{32} by weevils fed 10, 25 and 148 μc./ml. of solution. The average rates of uptake measured by the observed activity of the boll weevil are presented in table 2 and figure 1. A factorial statistical analysis of the biological data testing dose, sex, treatment and interactions between dose and sex, dose and day, and sex and day disclosed that there were no significant differences between any of these data. This could probably be attributed to the small numbers of weevils utilized in each treatment and the wide variation between individuals. However, the average values indicate a definite increase in radioactivity of the weevils proportional to the increase in radioactivity of the food solution.

In all cases where boll weevils were allowed to ingest P^{32}-labelled solution as adults, 46% or more of the observed activity was lost during the first 24 hours after removal from the radioactive food solution. The weevils fed 148 and 25 μc./ml. of solution lost approximately 50% of the activity during the first 24 hours, figure 1. Weevils fed 10 μc./ml. lost approximately 75% in 24 hours.

Another important aspect of the biological loss of P^{32} was the rate at which 90% of the exhibited radioactivity was lost. The weevils fed 10 μc./ml. of solution lost 90% of the radioactivity by the fourth day after removal from radioactive food. The weevils fed 25 and 148 μc./ml. of solution lost 90% of the exhibited radioactivity by approximately the eighth day. The radioactivity of eggs laid by these females for the first 10 days was very low and accounted for practically none of the P^{32} lost by the adults.

The data indicated that the radioactivity exhibited by the weevils upon emergence from larval diet containing various levels of radioactivity was proportional to the amount of P^{32} in the diet, table 3. There was little or no difference between sexes in exhibited radioactivity. When the average weight of the adults was taken into consideration, the males generally exhibited more radioactivity per milligram of body weight.

The rates of loss of radioactivity by weevils reared on radioactive diet was determined in the same manner as for the adults fed P^{32} in solutions and are presented in figure 2. These curves represent the daily loss of the radioisotope as c.p.m. corrected for decay.

These data indicated that the radioisotope was lost at a slower rate when the weevils were reared on a radioactive diet than when the radioisotope was fed in solutions to the adults. There was little apparent difference in the rate of loss of observed radioactivity by males and females.

The weevils reared in diet which contained 1.65 μc./g. lost 90% of the exhibited radioactivity between the 15th and 20th days. The weevils reared in 9.91 μc./g. of diet lost 90% of the exhibited radioactivity after approximately 25 days.

The weevils fed 10, 25 and 148 μc./ml. of solution orally retained a maximum of 2% of the initial level of radioactivity after 40 days. The weevils reared in larval diet to which P^{32} had been added, retained a minimum of 6% and a maximum of 8% of their initial radioactivity after 40 days.

BIOLOGICAL EFFECTS.—Results obtained of fecundity, longevity, preoviposition and oviposition period for boll weevils fed P^{32} in solution for 3 days immediately after adult emergence are given in table 4. None of the differences in these data were significant; however, there was

Table 3.—Amounts of radioactivity, per cent hatch and larval mortality of boll weevils reared in larval diet to which P^{32} was added.

Dose (μc./g.)	Per Cent Hatch	Larval Mortality	Males No.	Males Avg. C.p.m.	Females No.	Females Avg. C.p.m.	C.p.m./mg. Weevil Weight Male	C.p.m./mg. Weevil Weight Female
			Test No. 1					
Control	93.2	56.9	30	0	30	0	0	0
1.65	96.6	62.0	24	3,589	30	3,538	345.0	487.4
4.96	90.5	76.7	10	3,589	18	3,538	345.0	487.4
9.91	94.4	81.3	12	5,683	12	5,487	768.0	503.6
			Test No. 2					
Control	87.8	41.9	11	0	14	0	0	0
0.88	91.8	66.7	7	592	8	618	38.7	43.8
1.53	93.9	52.2	9	1,236	13	1,092	88.1	74.7
2.92	95.9	53.2	10	1,572	11	1,657	126.3	109.0
2.95	87.2	70.7	4	2,998	8	3,061	210.4	194.1
3.42	91.8	40.0	9	2,044	18	2,312	147.1	138.9
4.13	75.5	64.9	4	2,209	9	2,258	175.3	157.4

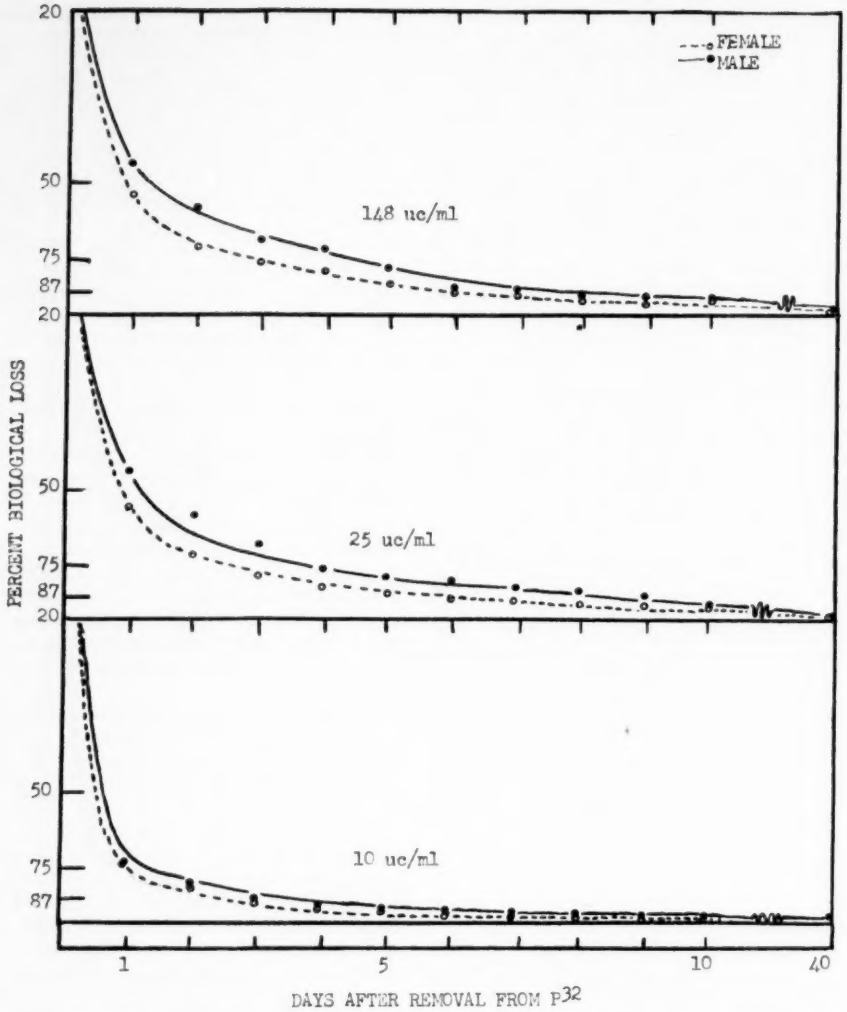

FIG. 1.—Rate of loss of P^{32} by boll weevils fed three concentrations of P^{32} labelled phosphoric acid in a honey-water solution for 3 days.

an indication of increased fecundity in the weevils fed 10 μc./ml. of solution.

Egg hatch and larval mortality obtained when boll weevils were reared in a larval diet which contained various concentrations of P^{32} are given in table 3. The P^{32} had no apparent adverse effects on egg hatch. The mortality of the larvae, however, increased as the radioactivity in the diet increased.

Fecundity, longevity, preoviposition and oviposition periods for boll weevils reared in a larval diet which contained P^{32} are given in tables 4 and 5. These data indicate that as the level of radioactivity was increased, the average length of life decreased compared with the control group. A highly significant interaction between dose and sex indicated that the effect on the females was greater than the effect on the males.

The difference in longevity between the sexes in the control group and the group reared in 4.96 μc./g. of diet was significant. The males reared at the low level of activity lived approximately 10 days longer than the females while those reared at the highest level of activity lived 12 days longer than the females.

Females reared in the two highest dosages of 9.91 and 4.96 μc./g. of diet did not lay eggs (table 4). Females reared from the lowest dose laid an average of 86.67 eggs per weevil compared with 138.1 eggs per weevil in the control group. Upon removing the variable of length of life from these data it was found that each female in the control group averaged 5.65 eggs per day of oviposition, while the females from the low dose averaged 4.96 eggs

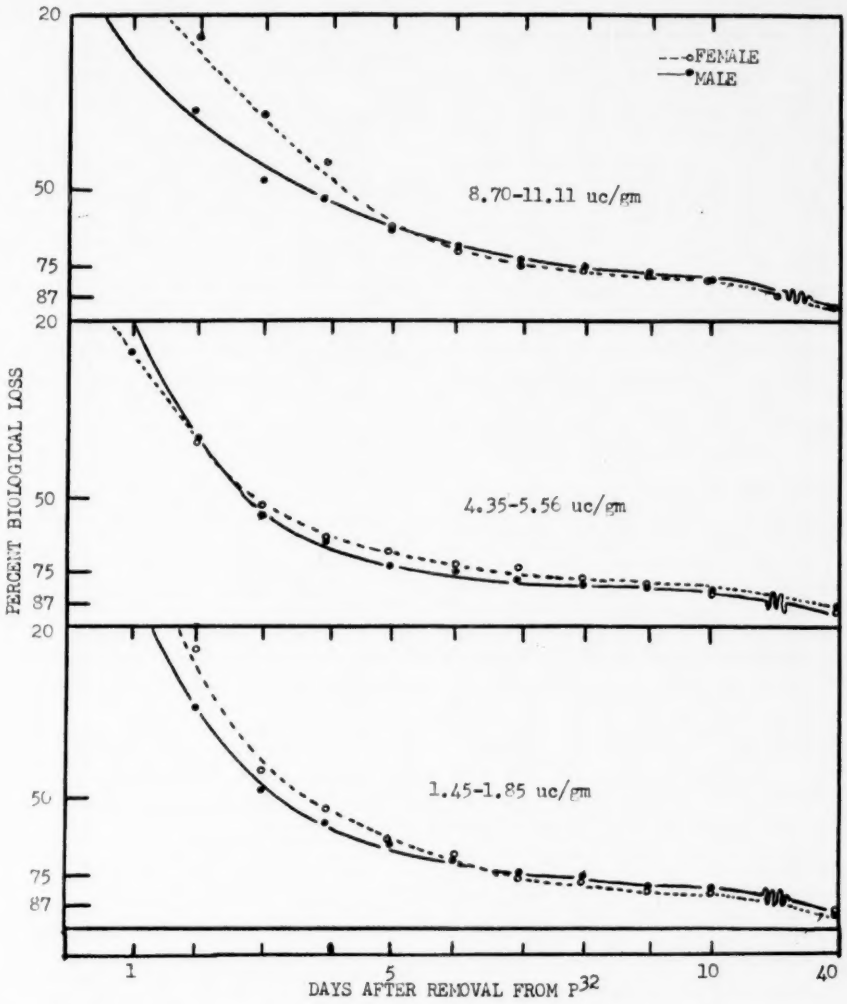

FIG. 2.—Rate of loss of P^{32} by boll weevils reared in larval diet which contained three concentrations of P^{32} labelled phosphoric acid.

Table 4.—The effect of P^{32} on longevity, pre-oviposition period, oviposition period and fecundity of the boll weevil when fed to adults in solutions and when added to the larval diet.

μc./ML.	NUMBER WEEVILS	TOTAL NUMBER OF EGGS	AVERAGE NO. EGGS PER FEMALE	AVERAGE NO. EGGS PER DAY	LENGTH OF LIFE (DAYS)		AVERAGE PERIOD (DAYS)	
					Male	Female	Preoviposition	Oviposition
			Fed to adults in solutions					
Control	7	961	160.2	4.4	43.7	41.7	6.2	36.5
10	6	1,070	214.0	5.7	42.6	44.0	6.2	37.8
25	5	991	198.2	6.3	41.3	37.2	5.8	31.4
148	7	1,135	162.2	5.1	44.0	39.0	7.0	32.0
			In larval diet					
Control	30	2,623	138.1	6.5	34.4	32.3	5.4	26.4
1.65	30	1,560	86.7	5.0	31.4	21.9	5.7	16.1
4.96	18	0	0	0	27.5	24.5		
9.91	12	0	0	0	25.3	13.8		

Table 5.—Cumulative mortality of weevils reared in larval diet with three levels of radioactivity.

Days After Removal	Per Cent Mortality							
	Control		1.65 μc./g.		4.96 μc./g.		9.91 μc./g.	
	Male	Female	Male	Female	Male	Female	Male	Female
1	0	0	0	0	0	0	0	0
2	0	0	0	0	25.0	0	0	20.0
3	0	0	0	0	25.0	0	0	30.0
4	0	0	0	0	25.0	7.7	0	30.0
5	0	0	0	0	25.0	7.7	0	30.0
10	0	0	6.3	11.1	25.0	33.1	0	40.0
15	0	0	6.3	27.8	25.0	40.8	12.5	50.0
20	5.3	17.6	18.8	44.5	25.0	56.1	37.5	70.0
25	21.1	23.5	25.0	66.7	25.0	63.8	62.5	80.0
30	26.3	35.3	43.8	72.2	50.0	71.5	62.5	100.0
35	42.1	47.0	56.3	77.8	50.0	71.5	87.5	—
40	42.1	70.6	56.3	88.9	50.0	86.9	87.5	—

per day of oviposition. The difference in this case was not significant. These data indicate that total egg production of females exposed to the low level of radiation was reduced only as a consequence of the shortened life of the female.

All males alive after 40 days from the two highest doses were removed to clean vials and mated with virgin females newly emerged from nonradioactive diet. All of these females produced viable eggs.

The observed radioactivity of eggs laid by female adults fed P^{32} in solution ranged from 0 to 50 c.p.m. per egg. The higher levels of activity in eggs were obtained while the observed activity of the adult was high and decreased very rapidly as the activity decreased in the adult. The activity of eggs laid by females reared on radioactive larval diet ranged up to 5 c.p.m., depending upon the activity of the female. There was no evidence of adverse effects on viability in these eggs with about 90% hatching. Observations on F_1 individuals produced from these eggs indicated no observed radioactivity and no apparent adverse effects on longevity and fecundity.

Discussion.—The female boll weevils did not consistently become more radioactive than the males when adults were fed P^{32} in solutions. Conversely, Babers et al. (1956), Blumel (1951), Bugher & Taylor (1949), Foott (1954), Hassett & Jenkins (1951), and others working with other insects found that the female became much more radioactive than the male. The female weevils emerging from the radioactive larval diet were seldom more radioactive than the males. The variation in observed radioactivity among individuals obtained in this study indicate that feeding activity of the individual may have determined the level of activity attained.

The effects of P^{32} on some biological functions of the boll weevil were generally more evident when the weevils were reared in radioactive larval diet than when adults were fed on radioactive solutions. Rates of 9.91 and 4.96 μc./g. of diet produced sterile females. The increase of mortality in these weevils was similar to the effects of gamma irradiation on other insects. However, Babers et al. (1956) found that house flies reared in a medium containing P^{32} were normal; and, that more adverse effects were produced when the flies were allowed to orally ingest P^{32} solutions than when reared on radioactive diet. Grosch & Sullivan (1952) found that ingested doses of P^{32} did not greatly decrease longevity in *Habrobracon* when fed ranges from 12.6 to 1445 μc./g. of food. In a later paper Grosch & Sullivan (1953) determined that *Habrobracon* could be sterilized by an oral dose of 200 μc./g. of honey and water. However, doses up to 148 μc./ml. of honey and water solutions fed to adults produced no apparent effects on fecundity and viability of eggs of the boll weevil.

In contrast to the report of Grosch & Sullivan (1953) that the majority of P^{32} was lost from *Habrobracon* in eggs during oviposition, the majority of P^{32} in boll weevils was not lost in eggs. Babers et al. (1956) postulated that there was a rapid loss of P^{32} from house flies by regurgitation during feeding. This possibility must not be overlooked when considering the possible mechanisms of loss of P^{32} by the boll weevil, although it seems more likely that most of the loss from boll weevils was by excretion.

It becomes apparent that loss of P^{32} in eggs was not the primary mode of loss by comparison of the curves showing the rate of loss by males and females, and females that were sterile. The rates of loss were similar in all instances. During the same period of time the excrement was more radioactive than the egg produced by an individual. These results indicate that excretion was one of the primary routes of loss. It may be possible that boll weevils may be followed in the field by location of radioactive deposits of excreta on or near the cotton squares.

After 40 days only the weevils reared in radioactive larval diet had readily detectable amounts of radioactivity remaining. However, the data given in table 1 indicate that oral doses in the range of 200 to 255 μc./ml. of solution would be feasible for relocation of boll weevils for 16 to 20 days in the field.

Feeding adults P^{32} in solution at the present time appears to be the better method of labelling boll weevils for field experimentation. This method assures less detrimental biological effects with a minimum of effort of preparation.

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Factors Involved in Differential Susceptibility of Corn Earworm Larvae to DDT[1]

R. T. GAST,[2] *North Carolina State College, Raleigh*

ABSTRACT

Full-grown corn earworms, *Heliothis zea* (Boddie), require more than 1,000 times as much DDT on a weight basis as small larvae in order to obtain LD-50 values for topical applications in acetone. Injected DDT in acetone is only slightly less effective on large larvae than on small ones. Chemical and radiometric analysis showed that lack of penetration by DDT through the integument of the large larvae was the chief factor causing the increase in LD-50 values. Addition of nonvolatile oils to the topically applied material increases the effectiveness of DDT to the larger larvae. Tests with different colored larvae indicate that light yellow larvae are approximately twice as susceptible as the dark red or black larvae.

Previous work (Gast 1959) had shown a 1,000-fold increase in the LD-50 values for topical applications of DDT on the corn earworm, *Heliothis zea* (Boddie), from small to full grown larvae. The LD-50 values were based on micrograms of insecticide per gram of larval weight. Many other workers have shown that larger larvae require greater amounts of insecticides to kill them than the smaller larvae but few of these workers based their work on a unit of weight of larvae. An excellent review was given by Mukerjea (1953) but to the author's knowledge no other insect has been shown to exhibit the 1,000-fold increase to insecticides during larval development as was found with *H. zea*.

This paper is a report of studies on the factors causing this decrease in the susceptibility of the larger *Heliothis zea* larvae to topical applications of DDT.

METHODS.—*Test Insect.*—Larvae of *Heliothis zea* were either collected from ears of field corn, or reared from eggs in the laboratory using tobacco leaves as a source of food. All field-collected larvae were held in the laboratory in separate containers for a period of not less than 3 days prior to treatment. Any larvae that exhibited evidence of disease, parasites, or other abnormalities were discarded. Twenty-four hours before testing the larvae were weighed to the nearest 100th of a gram, separated in groups to the nearest 10th of a gram, and held in separate containers without food until tested. The larvae used in the tests of color versus susceptibility were all collected from corn. The colors were arbitrarily selected as red, green, yellow, and black. There was a great amount of variation in *H. zea* with a continuous shading from one color to the next. Because of this only these four color extremes were chosen. In addition to selection on the basis of color, only worms weighing between 0.250 and 0.300 gram were used. Of the nearly 3,000 larvae collected, only 300 or 10% were considered as falling into one of the four color categories. Larvae used in the other tests reported in this paper were selected on a weight basis only without regard to color.

Insecticides.—The nonradioactive DDT was chiefly the p′ isomer obtained by three recrystallizations of technical material from hot ethanol. The melting point was 106.5° C. Radioactive DDT was synthesized by the author using chlorine 36 in the p-p′ position. After recrystallization, this material had a melting point of 106.3° C. When assayed on internal flow proportional counter the DDT gave 25 c.p.m./microgram.

Injection Studies.—Injecting lepidopterous larvae can be difficult owing to excessive bleeding at the site of the puncture. Menuson (1948) stated that cooling the larvae and injecting the material through the base of the proleg decreased bleeding. Preliminary trials showed a more satisfactory method was to anesthetize the larvae with CO_2 and then insert the needle on the lateral portion of the integument between the fifth and sixth abdominal spiracle. The needle was inserted 5 mm. parallel to the longitudinal axis of the larvae in order to avoid any internal organ. The CO_2 caused relaxation of the body wall muscles, and the larvae remained flaccid throughout the operation and for 30 seconds after the needle was withdrawn. Thus the wound was sealed by coagulated blood before the larvae regained the normal body tonus. Less than 0.5 microliter of blood was lost by this method. Acetone was used as the solvent. Tests showed no mortality from using injected amounts four times the highest rate used in the insecticidal tests. These larvae pupated and produced normal adults.

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[2] Present address: U.S.D.A. Boll Weevil Laboratory, Starkville, Mississippi.

In the insecticide evaluation tests, the DDT was dissolved in acetone and injected into each larva in proportion to its weight. Thus larvae weighing 0.1 gram would receive 1 microliter of solution while larvae weighing 0.7 gram would receive 7 microliters. At least four concentrations of DDT were used to determine the LD-50 values for each weight group of larvae. A minimum of 30 larvae was used to determine each point for the curves. After treatment, the larvae were held for 48 hours at 80° F. at which time mortality counts were made. Fresh food in the form of immature corn kernels was supplied fresh daily during the holding period. LD-50 values were determined by a probit analysis with fiducial limits set at 5% (Finney 1952). Only the nonradioactive DDT was used in these studies.

Topical Applications.—Two types of formulations were used in the topical application test. In one, the DDT was dissolved in purified acetone while the second type of formulation contained acetone and mineral oil. Both radioactive and normal DDT were used in the acetone formulations, while only normal DDT was used in the oil types. In all cases of topical application, the DDT formulation was applied to the dorsal part of the thorax of the larvae. The materials were applied to each larva in volumes of formulations proportional to its weight. Dosages were changed by changing the amount of DDT dissolved in the solvent. Formulations were applied at the rate of 25 microliters per gram of larval weight or 1 microliter for each 40 milligrams of larvae. A 10-microliter Hamilton Syringe with a 26-gauge needle was used for the applications. The plunger was driven by Gilmont dial face micrometer screw. Reproducible volumes could be obtained ± 0.10 microliter.

At least four points were used to establish dosage mortality curves and 30 or more larvae were used to establish each point. Treated larvae were held at 80° F. with fresh food supplied daily. Mortality counts were made after 48 hours, while chemical and radiometric analyses were made at 24 or 48 hours.

Analysis of Insects.—Extraction of DDT and DDE from the larvae was the same for both chemical and radio assay. The larvae were soaked in 10 ml. purified acetone for 1 minute and rinsed with an additional 5 ml. portion of acetone. The tip of the last abdominal segment was cut and viscera pressed out on a piece of filter paper by rolling a glass rod over the larvae from front to back. The integument was then ground with 5 ml. acetone and 1 gram anhydrous sodium sulfate. The viscera and blood on the filter paper were soaked in 5 ml. acetone for 24 hours, placed in fresh acetone and soaked an additional 24 hours. In all cases, the acetone was evaporated and the residue analyzed. The standard Schechter-Haller analysis was used for the chemical determinations. The radioactive DDT was determined in a Nuclear Measurements Model PC2 Proportional Counter using methane gas. The acetone solutions were evaporated directly on the planchets.

Counts made on the integument and viscera after the acetone extraction showed virtually no radioactivity, indicating that the extraction procedure was adequate. As a check on self adsorption by the sample, some larvae with known amounts of radioactive DDT were air-dried, ground with anhydrous sodium sulfate, and counted. Depending on the amount of material present on the planchet, not more than 5% loss of counts was due to self adsorption.

DDE was separated by use of an alumina-packed column and various solvents systems (Sternburg & Kerns 1952).

Table 1.—Effectiveness of injection and topical application of DDT to *H. zea*.

Larval Weight (g.)	LD-50 Values in Micrograms per Gram	
	Injected	Topical
0.00–0.09	—	42±6
.10– .19	3.5±1.8	70±33
.20– .29	1.2±1.1	71±34
.30– .39	1.8±1.2	589±209
.40– .49	2.5±1.5	4,170±1,720
.50– .59	8.0±3.6	>40,000
.60– .69	6.0±2.5	>40,000
.70– .79	9.2±2.8	>40,000

Results and Discussion.—The results of the injection studies using corn earworms are shown in table 1. A comparison is shown with results of a topical administration of DDT (Gast 1959).

Table 1 demonstrates that injected DDT is nearly as effective on large corn earworms as on small ones when the dose is computed on a microgram-per-gram basis. There was a slight increase in the amount needed to kill the larger larvae as compared with the smaller ones; however, there may have been other factors. Needles of the same size were used to inject both sizes of larvae, thus the injury to the smaller larvae would be proportionally greater than to the large larvae. While it is true that small larvae injected with plain acetone showed no mortality even at four times the rate used in the DDT applications, it is possible that this injury plus the DDT was more severe to the smaller larvae than to the larger ones. There also remains the fact that the smaller larvae may be more susceptible to the action of injected DDT than the larger larvae. In any event there was not the tremendous increase in LD-50 value as was experienced with the topical applications. There were two obvious explanations for this: the first one being that the larger larvae failed to absorb the DDT through the integument as rapidly as the smaller larvae; and second that the large larvae metabolized the DDT after it penetrated the integument before it could reach the site of action. Injection of large amounts of DDT directly into the blood would thus simply overwhelm the systems responsible for the metabolism of DDT.

The results of chemical analysis of larvae treated with DDT are shown in table 2. Only larvae still living at the end of the holding period were used in the analytical tests.

No attempt was made to distinguish between DDT and DDE in the chemical analysis. The results indicated that if the same sublethal amount of DDT is applied to larvae differing in size, essentially the same amount of material penetrates the integument regardless of size. In another test the DDT applied to the larvae was equal to the LD-50 for each weight group. The results are shown in table 3.

Table 2.—Analysis of *H. zea* larvae for DDT 24 hours after topical application.

Weight Group[a]	DDT Recovered (μg.)				
	Jar and Integument Wash	Integument	Viscera	Total	μg./g. in Viscera
0.10–0.19	35.1	63.2	0.5	98.8	3.5
.20– .29	29.2	68.6	0.7	98.5	2.9
.30– .39	24.8	72.0	1.6	98.4	4.6
.40– .49	30.1	66.4	1.4	97.9	3.1

[a] Ten larvae pooled for each weight group; 100 μg. DDT applied per larva.

Approximately 10% of the DDT was unaccounted for in both tests (tables 2 and 3). The chemical analysis used was for DDT only and it was possible that some of the missing material was in the form of DDE or other metabolites that would not respond to the method used.

A series of tests was next made using the radioactive DDT. The total recovery of DDT based on count of Cl-36 was not very much better than with the chemical methods (table 4).

The procedure for separating DDT from the DDE was subject to question since there was the possibility that some of the DDE found was due to breakdown by the alumina. The amounts of DDE recovered were also very small and the counts just slightly above background for the counter.

It was difficult to determine why there was a loss of DDT or its equivalent in the tests, since the analytical procedure would pick up the radioactive chlorine regardless of the compound it was in. Thus any metabolite of the DDT that contained the Cl-36 would be counted.

Table 3.—Analysis of *H. zea* larvae for DDT, 48 hours after topical application.

Weight Group[a]	DDT Applied per Larva (μg.)	DDT Recovered (μg.)				
		Jar and Integument Wash	Integument	Viscera	Total	μg./g. in Viscera
0.10–0.19	7.5	2.5	3.0	0.1	5.6	0.7
.20– .29	25	11	12.1	.3	23.4	1.2
.30– .39	175	101	55.0	1.6	157.6	4.5
.40– .49	2,250	2,100	120.00	2.0	222.0	4.4
.50– .59	5,500	4,947	386	2.1	4,335.1	3.8
.60– .69	6,500	5,892	402	2.5	6,296.5	3.9

[a] Ten larvae pooled for each weight group.

Table 5.—Effect of formulation on topical applications of DDT to *H. zea*.

Larval Weight (g.)	LD-50 Values of Formulations in Micrograms of DDT per Grams of Larval Weight		
	DDT +Ketone[1]	DDT+Oil +Ketone[2]	Oil +Ketone[2]
0.10–0.19	70±33	55±20	180±60
.20– .29	71±34	64±38	305±124
.30– .39	589±209	265±97	>40,000
.40– .49	4,170±1,720	783±306	>40,000
.50– .59	>40,000	4,509±1,200	>40,000

[1] Methyl-ethyl ketone at rate of 1 μl/40 mg. of larva.
[2] Solvent was made of 1 part white mineral oil to 2 parts methyl-ethyl ketone.

The alumina columns were assayed for activity after the separation and were found to contain none. Volatility was rather unlikely. The most plausible explanation for these losses was errors in technique.

Results of the radiometric and chemical test agreed quite closely as to the amounts of DDT absorbed by the viscera. Tests on the location of the DDT in viscera of the larger larvae indicated that the majority of the material was located in the fat bodies. Virtually none was found in the hemolymph. From the results of the analyses, it would appear that lack of penetration of DDT through the cuticle of the larger larvae was the chief reason that they were less susceptible to DDT than the small larvae. There was no evidence of any appreciable metabolism of the DDT by the larger larvae.

The DDT was applied in a ketone solution that evaporated rapidly, leaving a crystalline deposit of the DDT on the integument. Several different formulations were next tested using mineral oil in the ketone. This was nonvolatile and would allow for better penetration of the DDT through the integument. The results of these tests are shown in table 5 which show that the oil itself may have been responsible for the decrease in LD-50 value of the DDT-oil-ketone mixture for the smaller larvae. This fact is not too surprising, however, since mineral oil has long been recommended for the control of newly hatched corn earworm larvae in sweet corn. However, the data further show that oil had virtually no effect on the larger larvae. The DDT-ketone-oil formulation showed considerably greater toxicity to the larger larvae than the DDT-ketone formulation. One explana-

Table 4.—Radiometric analysis of DDT on *H. zea* 48 hours after treatment.

Weight Group[a]	DDT Applied per Larva (μg.)	μg. Recovered in						Total Recovered (μg.)
		Jar and Wash		Integument		Viscera		
		DDT	DDE	DDT	DDE	DDT	DDE	
0.10–0.19	7.5	3.8	0	3.1	0	0.1	0	7.0
.20– .29	25	9.5	0	12.4	0.1	0.1	0	22.1
.30– .39	175	97	0	60.2	0.0	0.9	0.1	158.2
.40– .49	2,250	1,800	0	202.0	0.2	1.7	0	2,003.9
.50– .59	5,500	4,753	0	429.0	0.3	2.5	0.4	5,185.2
.60– .69	6,500	5,920	0	380.0	0.2	2.4	0.3	6,302.6

[a] Ten larvae pooled for each weight group.

Table 6.—Susceptibility of different colored larvae of *H. zea* to topical applications of DDT.

LARVAL COLOR	NUMBER OF LARVAE	MORTALITY (%)
Yellow	78	64
Green	72	50
Red	75	42
Black	81	33

tion was that the DDT remained in solution in oil and thus penetrated the integument more readily than the crystalline DDT deposit left by the rapid evaporation of the ketone in DDT-ketone formulation. Another explanation could be that a synergistic effect between the oil and the DDT occurred, but this possibility seems rather remote. An attempt was made to analyze the internal absorption of the DDT in the DDT-oil-ketone formulation but satisfactory results could not be obtained.

During the course of the tests with *Heliothis zea*, it was noted that the lighter-colored larvae appeared to be more susceptible than the darker larvae. The results of a test to determine if such difference existed are given in table 6. Because of the limited number of larvae used, LD-50 values were not determined but instead all larvae were treated at the same rate.

Although the data are not suitable for statistical analysis, they indicate that the lighter-colored larvae are more susceptible to DDT than the darker ones. Preliminary analysis of the different larval groups indicate that the lighter-colored larvae absorb greater quantities of DDT through the integument than the darker larvae. Whether the lack of absorption by the darker larvae is due to a thicker cuticle or to the presence of pigments in the integument has not been determined.

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Dimethoate Absorption and Its Translocation and Distribution in the Cotton Plant[1]

JOSEPH HACSKAYLO,[2] D. A. LINDQUIST,[3] and T. B. DAVICH[3,4]

ABSTRACT

Cotton plants grown in sand culture treated with P^{32}-labeled dimethoate accumulated more insecticide in the leaves under environmental conditions which favored transpiration. Fruiting cotton plants treated with dimethoate as a soil drench accumulated relatively small amounts of the toxicant in the squares and bolls. Less than 1 microgram of dimethoate-equivalents was found in the stamens plus pistil of squares upon which the developing boll weevil (*Anthonomus grandis* Boheman) larvae feed.

Young cotton plants grown in dimethoate-treated nutrient solutions did not absorb the insecticide at the same rate they absorbed water. Also, plants grown in nutrient solutions deficient in phosphorus absorbed less insecticide than plants grown in a complete nutrient solution.

The systemic insecticide dimethoate is a solid at room temperature; however it is soluble in water (7% at 80°C.) and in many organic solvents (Anonymous 1959). Since the physical properties of this compound are unlike those of phorate and demeton, it was thought that its absorption by and distribution in plants might be different from those established for the above-mentioned insecticides. To understand plant systemic chemicals better, a knowledge of absorption, translocation, and distribution of compounds having various chemical and physical properties is essential. Work with demeton (Tietz 1954) and with phorate (Hacskaylo et al. 1961) has demonstrated that these materials are not absorbed by plants without producing some plant reaction to the toxicant. Also, there is a limit to the amount of insecticide a plant will tolerate. The present investigation was undertaken to study absorption, translocation, and accumulation of dimethoate in the cotton plant when the toxicant was supplied to the substrate.

MATERIALS AND METHODS.—Cotton plants of the Deltapine-15 variety were grown in nutrient solutions, sand, or soil in the greenhouse. A modified Hoagland's complete nutrient solution (Hacskaylo & Ergle 1955) was supplied to the plants growing in sand and solution culture. The tests were conducted either in the greenhouse or in a controlled climate cabinet (Behrens & Morton 1960). The P^{32}-labeled dimethoate[5] was used in all tests. All radioactive measurements were made with a thin end-window Geiger-Müller tube and a conventional scaling

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[2] Crops Research Division, Agric. Res. Serv., U. S. Department of Agriculture.

[3] Entomology Research Division, Agric. Res. Serv., U. S. Department of Agriculture.

[4] In cooperation with the Texas Agricultural Experiment Station, College Station, Texas.

[5] Obtained from Volk Radiochemical Co., 5412 N. Clark St., Chicago 40, Ill.

unit. Cupped stainless steel planchets were used to hold the samples assayed.

Effect of Temperature and Humidity on Accumulation of Dimethoate in Leaves.—Two cotton plants each were grown in 1-gallon cans filled with washed river sand and watered daily with nutrient solution. When the plants were 35 days old, eight cans (16 plants) were transferred to each of the following temperature and humidity conditions: 95° F. and 95% relative humidity (HTHH), 95° F. and 30% relative humidity (HTLH), 75° F. and 95% relative humidity (LTHH), and 75° F. and 30% relative humidity (LTLH). The plants were exposed to 1,800 foot-candles of light for 16 hours each day. After a 24-hour preconditioning period, 8 mg. of P^{32}-labeled dimethoate was added to each can as a drench. Five milliliters of acetone containing the toxicant was pipetted into about 100 ml. of water; this mixture was poured around the base of the plants in the cans. Calculations based on field capacity measurements of the sand showed that about 10 p.p.m. of dimethoate was present in the aqueous phase of each can.

The leaves from four plants in two cans were harvested from each holding condition 0.5, 1, 3, and 7 days after treatment. The leaves from each plant were handled individually, dried in an oven at 60° to 70° C. for 24 hours, and ground in a cutting mill to pass a 40-mesh screen. One hundred- to 200-mg. aliquots were radioassayed.

Distribution of Dimethoate in Fruiting Cotton Plants.—Four cotton plants growing individually in 1-gallon cans in the greenhouse were treated with 40 mg. of P^{32}-labeled dimethoate as a soil drench. The plants were about 60 days old and squares (flower buds), blooms, and bolls were present. These plants were held in the greenhouse and two plants each were harvested 2 and 8 days after treatment. The plants were divided into the following parts: old leaves, young mature leaves, and young leaves on the main stem, old leaves and young leaves on the fruiting branches, bracts from squares, blooms, and bolls, squares plus blooms, and bolls. These plant parts were dried, weighed, ground, and radioassayed as described above.

Two additional plants were treated with the insecticide as described above and 6 days later two squares and two 21-day-old bolls were removed from each plant. The squares were separated into peduncle, bracts, calyx, corolla, stamens, and pistils. The bolls were separated into peduncle, bracts, calyx, carpel, and lint plus seeds. These tissues were air-dried and radioassayed.

The seed cotton was harvested from open bolls of these plants 42 days after treatment with dimethoate. Ten seeds were radioassayed and the remaining planted in sand. A bioassay using the cotton aphid (*Aphis gossypii* Glover) as a test insect was conducted on the seedlings which emerged.

Relationship of Water and Dimethoate Uptake by Cotton Plants.—Six-week-old plants growing in nutrient solution were transferred individually to 250-ml. Erlenmeyer flasks which contained 220 ml. of nutrient solution with a concentration of 10 p.p.m. of P^{32}-labeled dimethoate. The plants were immediately placed on a bench in the greenhouse.

The original specific activity in the nutrient solution of each flask was measured by pipetting 2 ml. of the solution into a cupped planchet, evaporating the water slowly, and radioassaying the residue. Two plants were removed from the nutrient solution 2, 4, and 8 hours after treatment. The volume of water transpired by the plants and the specific activity of the remaining nutrient solution were determined.

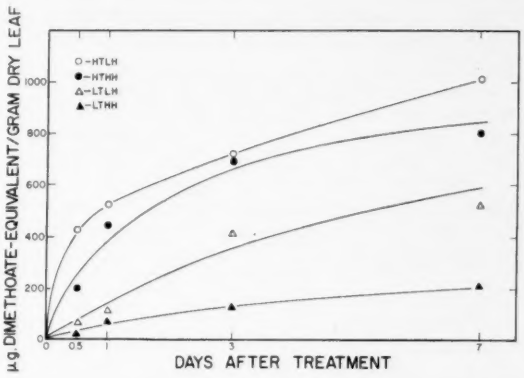

FIG. 1.—Effect of temperature and humidity on accumulation of dimethoate in cotton leaves. Plants grown in sand and treated with a drench of 4 mg. P^{32}-labeled dimethoate per plant. HT = 95° F., LT = 75° F., HH = 95% R.H., LH = 30% R.H.

Dimethoate Absorption and Translocation by Plants Grown in Complete and Phosphorus-Deficient Nutrients.—A greenhouse experiment was designed to compare absorption and translocation of dimethoate by cotton plants grown in nutrient solutions with adequate and deficient levels of phosphorus. Three-day-old cotton seedlings were transferred into nutrient solutions with and without phosphorus. When the plants were about 3 weeks old each group was transferred to complete nutrient solutions containing 10 p.p.m. of P^{32}-labeled dimethoate. Three healthy and three phosphorus-deficient plants were harvested 1, 3, 5, and 7 days after treatment. These plants were separated into leaves, cotyledons, stems, and roots; the plant parts were dried, weighed, ground, and radioassayed as described previously.

RESULTS.—*Effect of Temperature and Humidity on Accumulation of Dimethoate in Leaves.*—The accumulation of dimethoate in leaves of cotton plants treated with dimethoate as a soil drench and held under different temperature and humidity conditions is shown in figure 1. These data show that lower temperatures and higher humidities decrease both the rate of dimethoate accumulation and total amount of dimethoate accumulated during the 24 hours immediately after treatment. These same conditions have been shown to cause similar reductions in transpiration (Hacskaylo et al. 1961). However, 1 to 7 days after treatment, the rate of dimethoate accumulation in the leaves was nearly the same for plants held under the HTLH, HTHH, and LTLH conditions. The LTHH condition resulted in a slightly lower rate of accumulation than the other conditions 1 to 7 days after treatment and was nearly constant from 0 to 7 days. A similar experiment using phorate resulted in a nearly

Table 1.—Distribution of dimethoate-equivalents in cotton plants treated with 40 mg. of P^{32}-labeled dimethoate as a soil drench.[a]

Plant Part	Dimethoate-Equivalents per Gram Dry Tissue (μg.) at Indicated Days After Treatment		Distribution of Total Dimethoate Absorbed (%) at Indicated Days After Treatment	
	2	8	2	8
Old leaves on main stem	182	448	22	12
Young mature leaves on main stem	196	528	23	16
Young leaves on main stem	288	666	9	9
Old leaves on fruiting branches	200	585	32	42
Young leaves on fruiting branches	207	486	11	11
Bracts from squares, blooms, and bolls	58	140	2	9
Squares and blooms	20	—	1	—
Bolls	12	28	2	2

[a] Each value is the average for 2 plants.

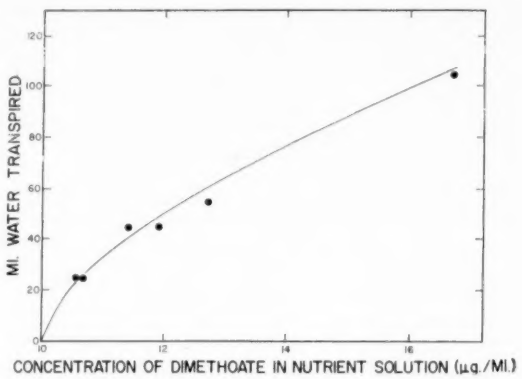

FIG. 2.—Relationship between water and dimethoate uptake by cotton plants exposed to 10 p.p.m. dimethoate in nutrient solutions.

constant rate of phorate accumulation from sand cultures (Hacskaylo et al. 1961).

Distribution of Dimethoate in Fruiting Cotton Plants.—The distribution of dimethoate in the cotton plant following soil drenching is shown in table 1. It is apparent that the compound is readily translocated to all leaves on the cotton plant. The young leaves on the main stems accumulated more dimethoate than other leaves on the basis of comparable weights; the bracts, however, accumulated less dimethoate than other leafy tissues. The squares and bolls accumulated less insecticide than other plant parts assayed. The reduced accumulation of dimethoate in the bracts, squares, and bolls was probably due to the lower transpiration of these structures.

The radioactivity in the various parts of squares and bolls is shown in table 2. Similar amounts of dimethoate-equivalent were found in all parts of the squares except the bracts which contained relatively large amounts. The stamens and pistil of a square, which the developing boll weevil (*Anthonomus grandis* Boheman) consumes, contained 0.8 microgram of dimethoate-equivalent. The bracts, carpel, and lint plus seed contained more radioactivity than the rest of the dissected bolls. All parts of the bolls contained more total radioactivity than comparable parts of the squares.

Table 2.—Distribution of dimethoate-equivalents in squares and unopened bolls 6 days after cotton plants were treated with 40 mg. of P^{32}-labeled dimethoate as a soil drench.[a]

Plant Part	Dimethoate-Equivalents (μg.)	
	Squares	Bolls
Peduncle	0.9	2.6
Bracts	11.3	20.4
Calyx	0.7	2.4
Corolla	0.6	—
Stamens	0.4	—
Pistil	0.4	—
Carpel	—	15.3
Lint plus seed	—	16.2
Total	14.3	56.9

[a] Each value is the average for 4 squares or bolls.

The cotton seed harvested from plants treated 42 days previously contained an average of 17 micrograms of dimethoate-equivalent per embryo. Negligible amounts of radioactivity were found in the seed coat and lint. There was no apparent aphid mortality observed on seedlings emerging from the seed described above. These results indicate that the radioactivity in the cotton seed was present in a nontoxic form. However, it was possible that toxic compounds might have been present in the embryos but were destroyed during germination.

Relationship of Water and Dimethoate Uptake by Cotton Plants.—Dimethoate and water were not absorbed by the plants from the nutrient solution in direct proportion (fig. 2). Although some dimethoate was absorbed by the plants, obviously the toxicant was being concentrated in the nutrient solution as transpiration continued. The data show also that more dimethoate was absorbed with the initial 30 ml. of water transpired than with subsequent 30-ml. portions. Following this initial absorption dimethoate was absorbed at essentially a constant rate of about 0.08 microgram per milliliter of water transpired. David (1951) reported that the roots of broadbean plants rejected schradan (a water-soluble insecticide) in an experiment similar to the one reported here.

Dimethoate Absorption and Translocation by Plants Grown on Complete and Phosphorus-Deficient Nutrients.—Data presented in table 3 show that plants grown in complete nutrient solution absorb and translocate more dimethoate than the phosphorus-deficient plants during a 7-day period. This was particularly evident in the leaves, since, without exception, greater quantities of the insecticide were found in the leaves of the plants grown on the complete than on the phosphorus-deficient solution. There appeared to be no consistent differences in the amounts of dimethoate found in the roots of either treatment.

DISCUSSION.—Preliminary experiments conducted at this laboratory have shown dimethoate to be a relatively poor systemic insecticide when applied as a cotton seed treatment. Severe phytotoxicity to cotton plants was observed when the chemical was applied as a seed treatment, soil drench, or foliar spray. Since dimethoate is

Table 3.—Absorption of dimethoate-equivalents by 3-week-old cotton plants grown on complete and phosphorus-deficient nutrient solutions.[a]

Harvest Time (days)	Dimethoate-Equivalents per Gram of Dry Tissue (μg.)							
	Leaf		Cotyledon		Stem		Root	
	1[b]	2[c]	1	2	1	2	1	2
1	270	180	180	120	60	40	140	130
3	500	390	390	330	80	60	190	200
5	780	720	570	730	90	80	290	310
7	1,010	860	770	500	110	110	450	430

[a] Each value is the average for 3 plants.
[b] Plants grown on complete nutrient solutions.
[c] Plants grown on phosphorus-deficient nutrient solutions.

soluble in water (up to 7% at 80° C.) and many organic solvents (Anonymous 1959), it was thought that the distribution pattern of this chemical within the cotton plant might differ from that of other insecticides, such as demeton and phorate, which have low water but high organic solvent solubilities. It was found, however, that dimethoate, like phorate (Hacskaylo et al. 1961), accumulated in greatest quantities in the leaves of cotton plants subjected to environmental conditions which favored transpiration. Only minor amounts of the total absorbed insecticide moved to the fruiting structures. The control of insects which feed on the fruiting forms seems highly doubtful since only 0.8 microgram was found in the stamens plus pistil of a given fruiting form.

The finding that 17 micrograms of dimethoate-equivalents per embryo accumulated in the seed collected 42 days after treatment would theoretically approximate a concentration of 170 p.p.m. of the insecticide. However, aphids placed on newly emerged seedlings from these radioactive seed were unaffected, indicating that very little or no radioactivity was in a toxic form or that the insecticide was rapidly converted to nontoxic metabolites during germination. It is probable that little or no insecticide is translocated to the seed in a toxic form.

The reduction in accumulation of dimethoate in the leaves of plants grown in solutions having no added phosphorus as compared with plants grown on complete nutrients was not surprising. Zhurbitskii & Wiei-Nan (1960), however, showed that corn plants grown in nutrient solutions low in phosphorus absorbed more $P^{32}O_4$ than plants grown in complete nutrients. It is apparent that a similar relationship does not hold for dimethoate accumulation by cotton plants deficient in phosphorus. It is noteworthy that the roots contained similar amounts of dimethoate whether the plants were grown in complete or phosphorus-deficient nutrients. The reduced amount of insecticide in the leaves of the phosphorus-deficient plants as compared with those grown in complete nutrient solution probably was associated with reduced transpiration.

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Incidence of Pink Bollworm Resistance to DDT[1]

William L. Lowry and Ching H. Tsao,[2]
Entomology Research Division, Agric. Res. Serv., U.S.D.A., Brownsville, Texas

Abstract

Laboratory tests in 1960 confirmed 1959 field observations that the pink bollworm (*Pectinophora gossypiella* (Saunders)) was resistant to DDT in certain areas of Mexico. Topical applications of DDT to moths originating at Tlahualilo, Durango, and Francisco I. Madero, Coahuila, resulted in LD-50's 13.6 and 7.4 times greater, respectively, than the LD-50 of moths originating near El Paso, Texas.

Large-scale field tests against the pink bollworm (*Pectinophora gossypiella* (Saunders)) in the Laguna of Mexico in 1946 indicated DDT was effective against this pest (Chapman et al. 1950). Since that time, DDT has been widely and effectively used for such control in both Mexico and the United States (Rude 1953, Hoyt 1951). Near the end of the 1959 cotton-growing season, L. B. Coffin[3] informed the authors that DDT apparently had failed to control pink bollworm in some localities near Torreon, Coahuila, Mexico. The reason for this failure was unknown, but resistance or increased tolerance to DDT was suspected. A series of laboratory tests designed to furnish more information on this subject was initiated

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[2] Now with the Department of Entomology, University of Georgia, Athens.
[3] Employee of the Plant Pest Control Division, Agric. Res. Serv., U.S.D.A., working in cooperation with the Defense Agricula de Mexico.

at Brownsville, Texas, in 1959 and continued in 1960.

MATERIALS AND METHODS.—Pink bollworm moths for the 1959 tests were obtained from three locations. Those from Torreon, Coah., Mexico and El Paso, Texas, were from open cotton bolls collected near the end of the 1959 growing season. Those from Brownsville were from bolls collected in 1958 and held in storage until November 1959. Infested material from the 1959 crop at Brownsville was not available because the pink bollworm control program requires stalk destruction before September 1.

Moths for the 1960 resistance tests were obtained from open cotton bolls collected near the end of the 1960 growing season in Mexico at Tlahualilo, Durango, and Francisco I. Madero, Coah., and in Texas at El Paso and Brownsville.

Moths that emerged from infested bolls placed in cages and kept under conditions favorable for pupation and emergence were collected daily. None of these collections were held longer than 3 days before being used in the tests. Acetone solutions of DDT were prepared from material containing 77% of p,p′ isomer. One microliter of solution was applied with a microapplicator to the thoracic sternum of each moth. Ten moths for each of the different dosages were treated per replicate. Moths treated with an equal amount of acetone were used as checks. The tests were replicated from three to six times and each replicate was initiated on a different date. Treated moths were kept in covered petri dishes without food and held at 80° ± 2° F. and approximately 60% relative humidity. Mortalities were recorded 24, 48, and 72 hours after treatment. The data given are based on average net mortalities 48 hours after treatment, calculated in accordance with Abbott's formula. Body weights were determined by weighing 5 to 10 lots of 10 moths each, or a total of 50 to 100 moths from each of the different locations.

Analysis of variance and regression lines were used to detect differences in resistance to DDT. Relative resistance of moths from the several different locations is expressed as the ratio of their LD-50's to that of El Paso moths which were least resistant.

RESULTS AND DISCUSSION.—There was no significant difference between mortalities of moths from the 1958 Brownsville and the 1959 Torreon material when an acetone solution containing 500 p.p.m. of DDT was applied to the moths. Forty-eight-hour mortalities were 51% and 38%, respectively. Nor was there any significant difference between mortalities of 1958 Brownsville moths

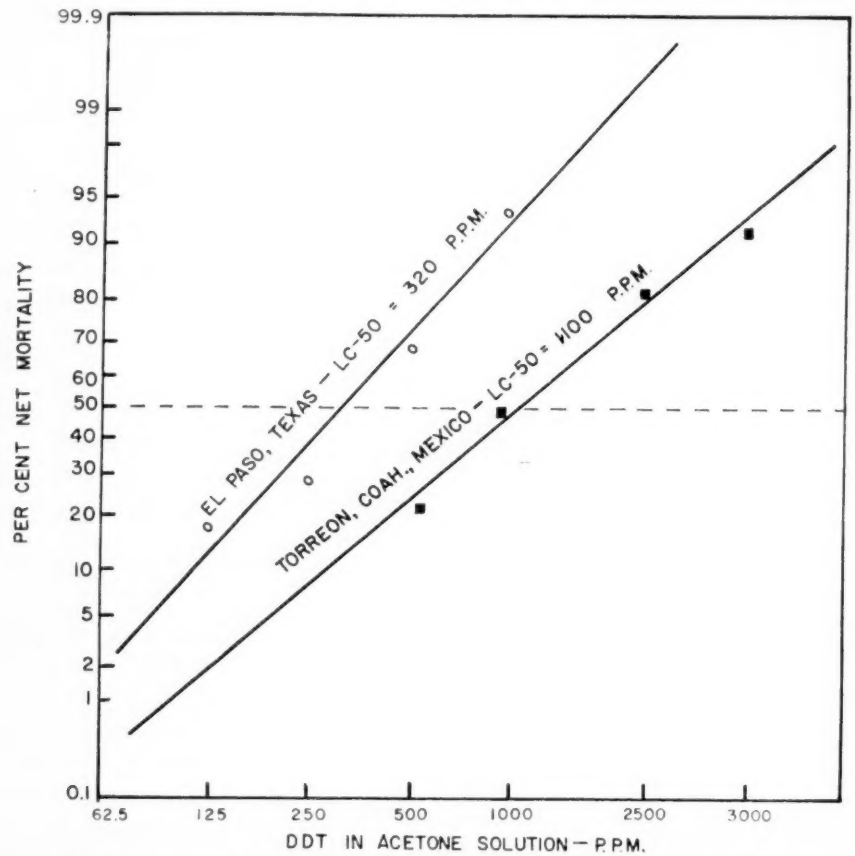

FIG. 1.—Mortalities and mean lethal concentrations of DDT for pink bollworm moths originating at El Paso, Texas, and Torreon, Coah., Mexico, 1959.

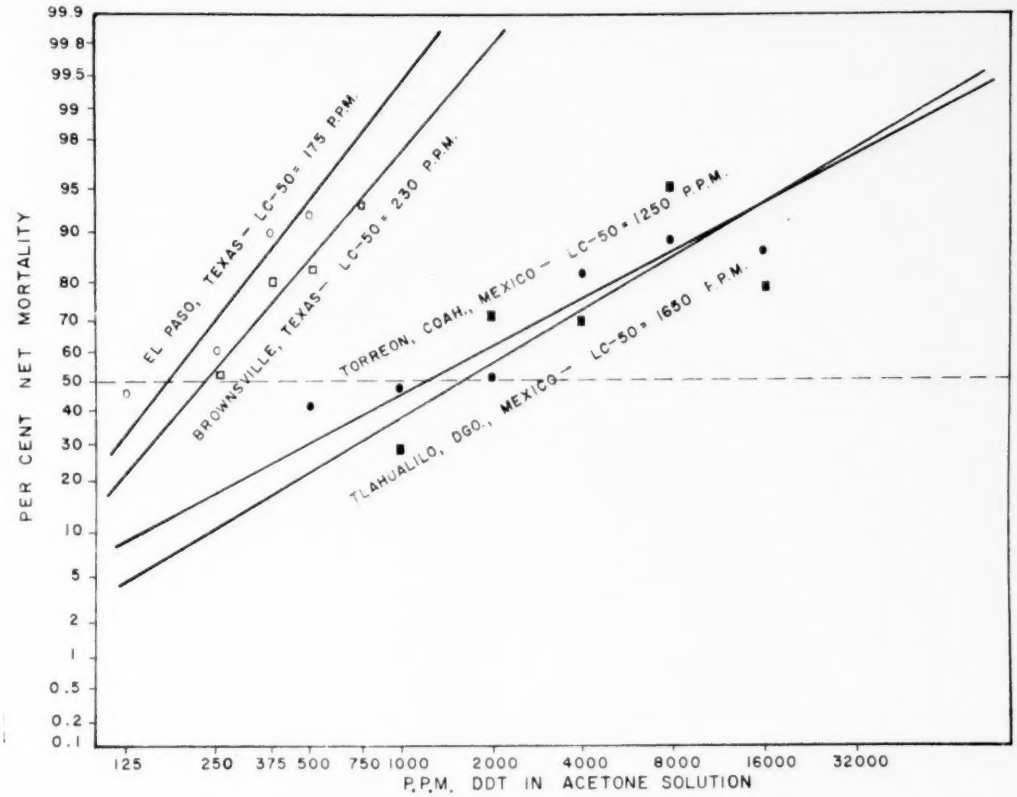

FIG. 2.—Mortalities and mean lethal concentrations of DDT for pink bollworm moths originating at several locations in Texas and Mexico, 1960.

and 1959 El Paso moths (64%). There was, however, a significant difference, at the 1% level, in mortalities of moths from the 1959 Torreon and El Paso materials.

The LC-50's of DDT-treated moths from the 1959 Torreon and El Paso materials were 1,100 and 320 p.p.m., respectively (fig. 1). On a body-weight basis, the LD-50's of moths from the same sources were 139.9 and 36.2 μg./gram, respectively (table 1).

Although these 1959 tests showed that moths from Mexico were more resistant than those from the other localities, the data left some doubt as to whether this greater tolerance was because of a natural difference between strains. A more positive indication of the development of resistance was to be found in later tests.

In the 1960 tests, moths from Mexico showed greater resistance to DDT than in 1959. Differences in resistance were compared on the basis of average amounts of DDT required for LC-50's when differences in average moth size and weight were not considered and for LD-50's based on body weights. Mean lethal concentrations are given in terms of p.p.m. of DDT in acetone solution (fig. 2). A summary of mean lethal dosages, average body weights, and relative resistance of moths from the several different locations is shown in table 1.

The results of these tests indicate that pink bollworm moths from at least some localities in the cotton-growing area surrounding Torreon, Coah., Mexico, are considerably more resistant to DDT than those from areas of the El Paso and lower Rio Grande Valleys of the United States.

Table 1.—Mean lethal dosages of DDT and relative resistance of pink bollworm moths originating at several locations in Texas and Mexico, 1959–1960.

ORIGIN	AVERAGE BODY WEIGHT (MG.)	LD-50		RELATIVE RESISTANCE
		μg./Moth	μg./g.	
1959				
El Paso, Texas	8.823	0.32	36.2	1.0
Torreon, Coah., Mexico	7.856	1.10	139.9	3.9
1960				
El Paso, Texas	9.248	0.175	18.9	1.0
Brownsville, Texas	10.149	.230	22.7	1.2
Francisco I. Madero, Coah., Mexico	8.977	1.250	139.2	7.4
Tlahualilo, Durango, Mexico	6.440	1.650	256.2	13.6

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Sticktight Flea Control on Chickens with Malathion Dust Self-Treatment[1]

J. L. Rodriguez and L. A. Riehl, *University of California Citrus Experiment Station, Riverside*

Abstract

Two applications of 4% malathion dust at an interval of 28 days in natural soil wallows or in prepared dust bath boxes at the rate of 15 pounds of dust per 100 chickens and by complete coverage of the floor area of pens at the rate of 1 pound of dust per each 20 sq. ft. gave control of the sticktight flea, *Echidnophaga gallinacea* (Westwood) for 150 days after the second application. Application in wallows or dust bath boxes required less malathion dust, only three-eighths the amount in range pens or three-fourths the amount in floor management houses, than the amount necessary for complete coverage of floor surface.

In southern California many poultry ranches are located near foothills with untilled land bearing sagebrush and other native vegetation. The ground squirrel, *Citellus beecheyi beecheyi* (Richardson), (Ryckman et al. 1954a,b) and other rodents living in the brushland are hosts for the sticktight flea, *Echidnophaga gallinacea* (Westwood). The flea may move from the rodents to flocks of chickens and turkeys kept in range pens. Occasionally the fleas attack hens in floor management houses with litter or young chicks in brooder houses.

Eads (1946) demonstrated excellent control of sticktight fleas on hens with 10% DDT. Linkfield & Reid (1958) used 5% malathion dust at the rate of 1 pound of dust per 20 sq. ft. of floor surface to control a light infestation of sticktight fleas on 1,500 hens in a floor management house.

Difficulties in control of sticktight fleas in southern California have caused economic losses to poultrymen. In addition, the attack by fleas of personnel as they go about their work is an irritating nuisance and carries the potential of plague. Good control of lice on chickens by self-treatment using malathion in dust bath boxes (Rodriguez & Riehl 1957) suggested trial of this method and chemical against the sticktight flea as a means of reducing treatment cost and improving control efficiency.

Materials and Methods.—A chicken ranch near Perris, California, was selected for the investigation. The tests were conducted during the spring and summer months. In 1957 and 1958 the fleas were found in the range pens. Each pen had an area of 8,000 square feet and held 1,000 White Leghorn pullets from 4 to 5 months old.

Wooden dust bath boxes (2 by 3 feet by 1 foot deep) (Rodriguez & Riehl 1957) were used in four pens; in each, a charge of 15 pounds of 4% malathion dust was used per box per 100 pullets. In four pens with many deep natural wallows these were used instead of the boxes; in each, a charge of 7.5 pounds of 4% malathion dust was used per wallow per 50 pullets, i. e., two wallows in place of one box. There were eight pens with only a few natural wallows; in four of these 4% malathion dust was applied to the total floor surface at the rate of 1 pound per each 20 square feet and in the other four at the rate of 1 pound per each 53 square feet. In each instance a second application of the treatment was made at 28 days.

Fig. 1.—A heavy infestation of sticktight fleas attached around the eye of an 8-day-old chick; this chick had more than 500 fleas feeding on the head and neck.

In 1959 and 1960, flea infestations were found on hens in floor management houses with straw litter 4 to 6 inches deep mixed with sand and dirt. Each house had an area of 800 square feet and held 200 hens and roosters. In four houses a dust bath box charged with 15 pounds of 4% malathion dust was put in the house for each 100 chickens. Four per cent malathion dust was applied to the total floor surface of four pens at the rate of 1 pound per each 20 square feet and of another four pens at the rate of 1 pound per each 40 square feet. In each instance a second application of the treatment was made at 28 days.

During the summer of 1960 a heavy infestation of sticktight fleas appeared suddenly on 5,000 eight-day-old chicks of the ranch and caused a serious stress to the chicks within several days. Figure 1 shows a chick with a heavy infestation of fleas attached around the eye; the chick shown had more than 500 fleas attached and feeding on the head and neck. Rapid control was necessary and

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4% malathion dust at the rate of 2 pounds per each 100 chicks was portioned equally in each corner of each brooder house. There were 500 to 1,000 chicks per brooder house. In each, the floor was covered with 1 to 2 inches of new rice hulls. A single brooder house with a relatively low infestation of fleas was maintained for 28 days as an untreated check when obvious damage by the fleas to growth of the chicks became apparent and made a treatment necessary.

The flea infestations in the respective treatment pens were measured by taking a sample of 10% of the chickens caught at random and counting the total number of fleas on the head, wattles and neck of each chicken. Determinations of flea infestation were made just before treatment and at intervals of each 7 days afterwards during the course of the experiment.

DISCUSSION OF RESULTS.— Data showing the performance of the various treatments are presented in table 1. Results of experiments in the range pens and the floor management houses have been combined. When a treatment occurred more than once a selection of data representative of the performance of the treatment was made.

Treating the natural soil wallows of range pens with 7.5 pounds of 4% malathion dust per wallow per 50 hens decreased the population of fleas rapidly but did not give complete control. By 14 days the flea population had started to recover; when buildup continued through 28 days the treatment was repeated. By 14 days after the second application the chickens were free of fleas with no recurrence for more than 50 days.

Dust bath boxes charged with 15 pounds of 4% malathion dust per box per 100 hens twice at an interval of 28 days controlled the fleas for 150 days.

In the range pens dusting the entire surface of the floor with 1 pound of 4% malathion dust per each 53 sq. ft. of area required the same total amount of dust as that used for the wallows and the dust bath boxes but a double application to the floor with a 28-day interval did not control the fleas. Increasing the dosage to 1 pound per 40 sq. ft. in floor management houses was only little better. Covering the floor at the rate of 1 pound per 20 sq. ft. gave complete control following the second application but in range pens this required 2.5 times as much malathion dust as was used in the wallows and boxes.

In the brooder house, application of 2 pounds of 4% malathion dust per 100 chicks portioned equally in each corner of the house gave complete control of the fleas by the seventh day. The chicks remained free of fleas until observations were discontinued at 60 days when the chicks were transferred from the brooder house to grow pens. Although the chicks were very young there was no indication that the malathion dust treatment had any adverse effect on their development and growth.

In the range type pens if there was a sufficient number of wallows to permit the proper ratio to chickens it was preferable and more efficient to put the malathion dust in the wallows as this made it possible to save the cost of the wooden boxes for dust baths. Comparable control was obtained with the same amount of malathion dust. The chickens were accustomed to dusting themselves in the wallows. However, if the number of wallows is insufficient the box is the method of choice for the dust bath.

Table 1.—Control of the sticktight flea on pullets in range type pens and on hens in floor management houses with litter using 4% malathion dust in the natural soil wallows, dust bath boxes or on the entire floor surface.[a]

	Treatments					
	Range Pen		Floor Management House			
		Natural Soil Wallow	Dust Bath Box	Total Floor Surface Application		
Days	Untreated	7.5 Lb./Wallow/50 Hens	15 Lb./Box/100 Hens	1 Lb./20 Sq. Ft.	1 Lb./40 Sq. Ft.	Untreated
			Average Number of Fleas Per Bird			
0	75	64	600	550	675	670
7	76	0.8	200	23	14.4	530
14	39	7.0	—	—	—	—
21	—	—	20.6	50	280.0	800
28	48	12.6	—	—	—	—
			Treatments Repeated			
7	65	0.6	2.6	0.5	11	675
14	60	0.0	.3	1.2	150	800
21	—	—	.6	.9	300	239
28	78	0.0	.4	0	240	738
35	—	—	0	0	241[b]	938
42	—	—	0	0	—	960
50	83[c]	0.0[c]	—	—	—	—
60	—	—	0	0	—	838[d]
120	—	—	0	0	—	—
150	—	—	0	5	—	—
160	—	—	20	10	—	—

[a] Each range pen held 1,000 pullets and had an area of 8,000 sq. ft. and each floor house held 200 hens and had a floor area of 800 sq. ft.
[b] Study terminated.
[c] Birds moved to wire cages.
[d] Control treated.

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Seasonal Occurrence of Diapause in the Boll Weevil in Mississippi[1]

E. P. LLOYD and M. E. MERKL, *Entomology Research Division, Agric. Res. Serv., U.S.D.A.*[2,3]

ABSTRACT

Initial entry into diapause by segments of populations of the boll weevil (*Anthonomus grandis* Boheman) were studied in one field in 1958 and in seven fields in 1959. In the 1958 field diapause occurred by August 26. In the 1959 fields it was observed as early as August 11 and as late as September 8. Weevil populations generally entered diapause in two distinct periods. Gross observations indicated that entry into diapause in the first period was related to the maturity of the plants in the different fields. An adequate food supply appeared to be necessary.

Diapausing weevils were first recovered from ground trash on September 10 in 1958 and September 9 in 1959, which indicated movement by diapausing segments of the weevil populations to overwintering quarters well in advance of the first killing frost.

Recent studies have demonstrated that the boll weevil (*Anthonomus grandis* Boheman) enters diapause prior to seeking overwintering sites in the late summer and fall. It has been generally accepted that this insect hibernates as an adult during the winter months. Boll weevils are commonly recovered from ground trash of forested areas adjacent to cotton fields in central and northern Mississippi. They have also been observed overwintering in Spanish-moss (*Tillandisa usneoides* L.) in other areas.

Brazzel & Newsom (1959) reported that this insect undergoes diapause instead of becoming inactive as a response to low temperatures during the fall and winter months. Diapause in the boll weevil, as reported by these authors, is characterized by cessation of gametogenesis and atrophy of gonads, increase in fat content, decrease in water content, and decrease in respiratory rate. They observed evidences of diapause in one cotton field in Louisiana as early as July 30 but in another field not until September 24. Brazzel & Hightower (1960) showed that a segment of the boll weevil population went into diapause in late July in fields of dry-land cotton in central Texas. However, this phenomenon did not occur in heavily fertilized, irrigated fields in this locality until September.

The work reported here was undertaken to determine the seasonal incidence of diapause in the boll weevil in two areas of Mississippi. Observations made during this study also serve as a starting point for ascertaining the stimuli that induce weevil entry into diapause under field conditions.

METHODS.—The cotton fields selected for this study were in the delta and hill areas. Samples of adult weevils were collected by hand from squares, blooms, bolls, and vegetative parts of the plants. Collections were made at weekly intervals in the respective fields beginning the last week in July and continuing until frost. The field sampled in 1958 was located in the hill area at Duck Hill, and of the seven fields sampled in 1959, four were in the hill area and three in the delta area.

Measurements taken as criteria of diapause were of fat and moisture content and degree of atrophy of the reproductive systems. Random samples of 75 to 100 weevils were collected by hand at weekly intervals from each field beginning in July and ending November 4. Twenty-five to 50 of these weevils were sexed and dissected for evidences of diapause. The characteristics used to distinguish diapausing weevils were the same as those used by Brazzel & Newsom (1959). Weevils considered to be in the firm state of diapause were as follows: (a) Females: ovaries atrophied, no eggs present, and visible fat; (b) males: testes atrophied, no sperm bundles present, and visible fat. Weevils considered to be in the intermediate stages of diapause were as follows: (a) Females: ovaries reduced in size but longer than 2.0 mm., some eggs present, and some visible fat; (b) males: testes reduced in size but larger than 0.6 mm. in diameter.

Samples of 50 weevils were used each week to make moisture and fat determinations. The live weevils were weighed, then killed and dried for 24 hours in an oven at 70° C., and reweighed to obtain dry weights and percentage of moisture. Fat content was determined chemically as ether soluble extractions and expressed as a percentage of the dry weight.

Ground trash was sampled in the wooded areas surrounding the 1958 field and three of the 1959 fields. Collections were made during the periods July 29 to November 12 in 1958 and September 2 to November 3 in 1959.

RESULTS.—*1958 Study.*—Overwintered weevils infested the field used in 1958 during the pre- and early squaring periods. First-generation weevils punctured

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approximately 90% of the squares. Seven weekly applications of gamma BHC-DDT dust (3:5) were applied to this field at the rate of 8 to 12 pounds per acre with tractor-mounted equipment. These applications were started for control of first-generation weevils. Two of these applications applied late in the morning were considered to be ineffective because of thermal convective air currents that resulted in drift of the insecticide.

The initial appearance of diapause in the field population occurred on August 26 at the end of the fruiting period. Diapausing weevils were collected each week thereafter until frost. The moisture content of the weevil samples decreased but the fat content increased with the appearance of diapausing weevils in the population. Results are shown in table 1.

1959 Study.—The first of the three delta fields studied in 1959 was located at Greenville (field 1). It received no insecticide treatment during the season. The field contained two distinct plantings of cotton and received no fertilization.

The second at Winterville (field 2) was grown under conditions typical in this area. The cotton was heavily fertilized and treated with methyl parathion-DDT spray throughout the season, with the last application on September 8. The field was defoliated on September 17, which caused most of the squares and small bolls to abscise. A desiccant applied October 15 did not effectively kill the plants.

In the third located in Sunflower County (field 3) cultural practices were similar to those noted in the second. This field was treated regularly with methyl parathion-DDT until defoliated on September 16.

Diapausing weevils were first collected in field 1 on August 31. This field contained two plantings. Most of the weevils apparently moved from the first planting, which matured early in August, to the second planting. Diapausing weevils did not appear then. However, when the second planting reached maturity, weevils in the intermediate stages of diapause did appear. Initial entry into diapause was observed in fields 2 and 3 on September 8. These two fields were highly fertilized and late in reaching maturity compared with field 1 and fields 4, 5, 6, and 7.

A field at Satartia, Yazoo County (field 4) had two plantings. The first, planted during the first week in April, produced the oldest cotton in the study and was heavily infested with weevils throughout the fruiting period. Weekly applications of methyl parathion-DDT spray were begun with the emergence of first-generation weevils and continued until mid-August.

Three other fields were studied at Duck Hill (fields 5, 6, and 7). Field 5 received no insecticide treatment during 1959. It was heavily infested with weevils throughout the season; 95% of the squares were punctured after mid-July. Field 6 was very similar to field 5 except that it received weekly applications of gamma BHC-malathion-DDT (3:5:5) dust from mid-July until the last week in August.

Production practices commonly used in the delta area were employed in field 7. This highly fertilized field received regular applications of methyl parathion-DDT spray, with the last application on August 26.

A weevil in the intermediate stages of diapause was collected in field 4 on August 11, the earliest date recorded in 1959 for such an appearance. This planting was also the earliest in the seven fields studied in 1959. The plants reached maturity in early August. Segments of the weevil population to enter diapause next were found in field 5 on August 19. This field, with a low level of soil fertility, received no insecticide treatment during the season. Plants reached maturity in mid-August. Fields 6 and 7 in the hill area had diapausing weevils present the following week on August 26. The occurrence of this phenomenon was coincident with plant maturity and termination of the insect-control programs. Results obtained in the 1959 fields are given in table 2.

Table 1.—Seasonal incidence of diapause, fat and moisture content, and hibernating weevils in diapause in a population in and around a field treated with gamma BHC plus DDT (3:5) at Duck Hill, Miss., 1958.

Date Collected	Number of Field Weevils in Diapause (%)	Moisture Content (%)	Fat Content[a] (%)	Number of Diapausing Weevils in Ground Trash	
				Females	Males
July 29	0	63.0	10.3	0	0
Aug. 12	—	60.6	16.0	—	—
26	4	—	—	0	0
Sept. 10	36	—	—	1	0
16	40	49.6	34.3	1	1
24	60	52.4	34.0	0	1
30	70	51.2	23.9	2	1
Oct. 8	56	50.3	23.6	1	1
14	58	48.4	26.3	5	1
22	37	54.4	19.4	1	0
29	26	54.0	21.8	7[b]	8[b]
Nov. 5	22	51.9	17.8	5	12
12	—	—	—	0[c]	5[c]

[a] Based on dry weight.
[b] 7 and 8 reproductive females and males also found.
[c] 7 and 6 reproductive females and males also found

DISCUSSION.—*Field Examinations.*—The first appearance of diapausing weevils differed from field to field and was apparently related to the time the plants reached maturity. This finding agrees with that of Brazzel & Hightower (1960) in Texas. They postulated that some interaction of the insect-host plant relationship must exist.

The boll weevil populations entered diapause in two general periods. The first occurred when the plants in the different fields reached maturity. The second was observed to occur in late September or early October. The reasons for this second rather distinct entry were not readily apparent in either 1958 or 1959. Brazzel and Hightower also observed this second entry period and attributed it to the lower temperatures in late September.

The available food supply appeared to be an important factor in the time required for weevils to accumulate sufficient fat to survive the winter. In field 1 of the 1959 tests, regrowth during September and October on the earlier of two plantings provided the population with an excellent food supply. Weevil entry into diapause, as shown in figure 1, was considered to be optimum with no distinct lag in the percentage of diapausing weevils be-

Table 2.—Seasonal incidence of boll weevil diapause (total per cent in intermediate and firm diapause) in seven fields in Mississippi, 1959.

Week Beginning	Field Number[a]						
	1	2	3	4	5	6	7
July 26	—	0	0	0	0	—	—
Aug. 2	0	0	0	0	0	—	—
9	0	0	0	4	0	0	0
16	0	0	0	4	0	6	0
23	0	0	0	36	44	24	8
30	8	0	0	52	32	40	20
Sept. 6	72	4	4	80	88	60	72
16	56	20	24	40	44	44	32
20	68	20	60	40	36	16	24
27	56	52	48	52	20	32	20
Oct. 4	64	24	32	68	20	16	16
11	64	8	16	68	56	44	40
18	76	44	16	72	36	20	40
25	48	52	28	60	36	40	28
Nov. 1	72	52	28	48	28	40	—

[a] Fields 2, 3, 4, and 7 treated with methyl parathion-DDT, field 6 treated with gamma BHC-malathion-DDT; fields 1 and 5 were untreated.

tween the first and second periods. In contrast, field 7, which was effectively defoliated in early October, contained only a small percentage of weevils that reached the firm state of diapause in the second period. The two periods of entry were rather well defined in the other five fields. The increase in the percentage of weevils entering diapause was correlated with second growth on the plants in each of the fields.

Boll weevil moisture and fat determinations made during the experiment provided valuable supporting data in this study. Table 1 and figure 1 provide representative data obtained during the experiment. The initial appearance of diapausing individuals in each population normally was accompanied by an increase in fat content (based on dry weight) and a decrease in the body-moisture content. When the percentage of diapausing weevils in the field was highest, the fat content was highest and the moisture content lowest. In general, when the fat content was 10% or less, and the moisture content ranged from 65% to 70%, the population contained 100% reproducing weevils. With the appearance of diapausing weevils in the field populations, the fat content increased to a range of 23% to 34% within 2 or 3 weeks. The moisture content decreased to a range of 48% to 56% when the diapausing population reached its maximum numbers prior to entering hibernation quarters.

Ground Trash Examinations.—These examinations around the field studied in 1958 yielded the first diapausing weevil on September 10 and additional weevils in each of the subsequent weekly examinations, which were made until frost (table 1). Weevils obtained prior to October 29 were fat and considered to be in diapause. However, on October 29, 15 of 30 recovered from ground trash were considered to be reproductive. A light frost occurred on October 28 that apparently caused the reproductive weevils to enter the ground trash. The following week 17 weevils in diapause were collected in ground trash. These

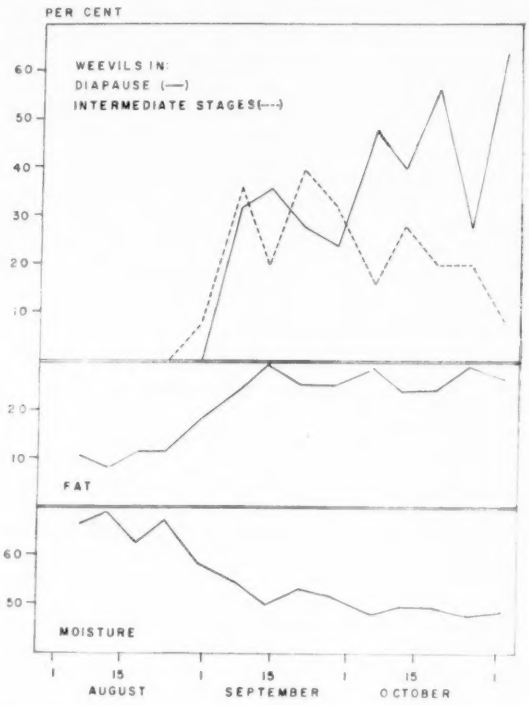

FIG. 1.—Seasonal incidence of diapause and fat (based on dry weight) and moisture content of a boll weevil population in an untreated field at Greenville, Miss. (field 1). 1959.

limited data indicated that the reproductive weevils found the previous week had either died or returned to the field. Following a killing frost on November 12, 18 weevils, 13 of them reproductive, were collected from ground trash. This second observation seemed to substantiate the earlier record, which indicated reproductive weevils might be temporarily forced into ground trash by freezing or near-freezing temperatures. No reproductive weevils have been observed to survive the winter in ground trash.

The first weevil collected in 1959 from ground trash adjacent to cotton fields was taken on September 9 at field 6. Weevils were collected each week thereafter through November 3. Weevils were collected initially around fields 4 and 7 on September 22 and 23, respectively. Subsequent trash examinations, terminated following frost, yielded diapausing weevils at weekly intervals. These data clearly indicate that segments of the boll weevil population entered diapause and sought suitable overwintering quarters in Mississippi as early as September 9 during the 2-year period of this study.

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Control of Certain Potato Insects in Arizona with Soil Applications of Granulated Phorate[1]

PAUL D. GERHARDT and DON L. TURLEY, *University of Arizona, Tucson, Arizona*

ABSTRACT

The principal insect pests of potatoes in Arizona requiring control are the potato psyllids, green peach aphids, leafhoppers and thrips. Of these the most serious pest is the potato psyllid, *Paratrioza cockerelli* (Sulc).

A soil application of granules containing 10% phorate at the rate of 20 pounds per acre (2 pounds actual) made to Red Pontiac and Kennebec potatoes at time of planting gave good control of the potato psyllid and green peach aphid (*Myzus persicae* (Sulzer)). Granules were applied approximately 2 inches below the seed piece and 4 to 5 inches to the side in the fertilizer band on one side only.

The young plants did not readily pick up the phorate from the soil until after the first irrigation. Effective insect control was obtained for approximately 100 days after planting. A fertilizer-phorate mixture (0.2% phorate) applied to both sides of the seed piece also gave satisfactory control of psyllids and aphids. An aphid buildup may occur late in the growing season as the effectiveness of the phorate diminishes. Lack of psyllid control was found to reduce the potato yield by 50% or more.

The three major potato-producing areas in Arizona are: the Salt River, Casa Grande, and Yuma Valleys. One of the most important insects affecting this crop is the potato psyllid, *Paratrioza cockerelli* (Sulc), which often causes serious losses if not controlled. Other insects generally requiring control are the green peach aphid, *Myzus persicae* (Sulzer), leafhoppers of the genus *Empoasca*, and thrips, *Frankliniella occidentalis* (Pergande). To control these insects, an early application of sulfur dust combined with parathion or other appropriate insecticides is recommended. Subsequent dustings with this or other insecticides should be given as needed. Later in the growing season, sprays may be used in place of dusts for aphid control. Insecticides are usually applied by air, although early in the season some growers may use ground equipment.

REVIEW OF LITERATURE.—It has been shown that systemic insecticides are effective in the control of many sucking insects on a wide variety of crops. Hopkins et al. (1959) have shown that when phorate was applied as a side dressing to cotton, it decreased the number of cotton aphids for 15 months. It was also found that oats, planted in rotation with phorate-treated cotton, had fewer greenbugs. Landis & Schopp (1958) applying systemic insecticides as sprays, were able to control the green peach aphid on potatoes. Dobson (1958) using granular systemics controlled spotted alfalfa aphids on alfalfa.

Andres et al. (1959) using systemic insecticides applied by various methods controlled the cabbage aphid on cabbage and cauliflower. Effective aphid control on cabbage was obtained for more than 26 days when a granular formulation of phorate was applied as a side dressing, in either the seed or transplant row.

Getzin & Chapman (1960) found that plants absorbed greater amounts of phorate from sandy soils than from heavier soils. Knoke (1959) obtained insect control on potatoes up to harvest time with systemic insecticides. His results showed that treatments with granules were more effective than sprays.

More recently Bacon (1960), working with potatoes in California, found that a single application of phorate granules at planting time would control the green peach aphid on the spring potato crop for 80 days. He also found that for good control phorate granules should not be used at less than 2 pounds actual phorate per acre.

METHODS AND MATERIALS.—In view of results obtained by earlier workers, preliminary trials to evaluate certain systemic insecticides were started in Arizona in 1959. In these trials potatoes were treated with a fertilizer-phorate mixture which contained a 2% phorate granule formulation and was applied at 1,200 pounds per acre or 2 pounds of actual phorate. These trials indicated that one application at planting time controlled aphids throughout most of the growing season.

In 1960, several tests were set up using a 10% phorate granular formulation to control the green peach aphid, potato psyllid, and thrips. The materials included 10% phorate granules, and 2% parathion+50% sulfur dust. In one of the tests 1,000 pounds per acre of a 10-20-5 fertilizer, including 0.2% phorate, was applied.

All applications of phorate granules were made with a Gandy dry-insecticide applicator, mounted on the front of an Iron-Age or John Deere two-row potato planter. The fertilizer bands were placed in the normal position, 4 to 5 inches to each side and 2 inches below the seed pieces. The 10% phorate granules were placed in one of these fertilizer furrows, approximately 20 pounds (2 pounds actual) per acre.

Where 2% parathion+50% sulfur dust was used it was applied by airplane at approximately 30 pounds per acre. One field received an application of a mixture of 0.2% phorate and 10-20-5 fertilizer, at the rate of 1,000 pounds per acre, amounting to 2 pounds of phorate per acre. In this case the phorate-fertilizer mixture was placed on each side of the seed as described above.

Two varieties of potatoes were planted: Red Pontiac, a red-skinned potato; and Kennebec, a smooth white-skinned potato. Only one test was conducted with the Kennebec, a chipping variety. All plantings were made in February and early March, and were harvested in June and July.

TEST NO. 1—MESA BRANCH STATION.—The Red Pontiac variety was planted February 16. All tests were either paired plot or randomized block design and treatments were replicated five times. The treated plots re-

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Table 1.—Control of green peach aphid and potato psyllid on Red Pontiac potatoes, Mesa, Arizona, 1960.

Sampling Dates	Number of Insects per 25 Leaves			
	Untreated		Treated[a]	
	Green Peach Aphids	Psyllids[b]	Green Peach Aphids	Psyllids[b]
3/24	2,829	0	2,473	0
4/6[c]	64	0	45	0
4/15	134	0	14	0
4/26	55	55	1.3	0
5/6	8	65	0	0
5/13	3	6	0	0
5/25	0.3	0.5	0	0

[a] Planted and treatment applied February 16.
[b] Only psyllid nymphs were counted.
[c] First irrigation on April 1.

ceived 20 pounds per acre of 10% phorate granules at planting time. Prior to the first irrigation after planting and when the plants were 2 to 3 inches tall, a very high population of apterous green peach aphids was present.

Table 1 shows the results of insect counts taken at weekly intervals from March 24 to May 25. The initial count was taken March 24 prior to the first irrigation on April 1. The untreated and phorate-treated plots showed a high population with counts of more than 403 aphids per leaf. After this irrigation the aphid abundance dropped in both treated and untreated plots but the count of April 15 indicated a continued drop in numbers of aphids in the treated plot, while they were building up again in the untreated plot.

In mid-April eggs and nymphs of the potato psyllid were found in the untreated plots. Approximately 7 to 10 days later, the psyllid-infested plants developed symptoms of psyllid yellows which gradually became more severe. No psyllids or symptoms of psyllid yellows were found in the phorate-treated plots. The yields from these plots, shown in table 2, are given in 100-pound sacks per acre of the various U. S. grades. The phorate-treated plots yielded a higher percentage of No. 1-A's and No. 1-Jumbos, and a lower percentage of No. 1-B's. This result probably indicates the effect of psyllid control by phorate. Figure 1 illustrates the effect of potato psyllids on early tuber set and development.

TEST NO. 2—QUEEN CREEK, ARIZONA.—In this test, the Kennebec variety was planted February 6 in an 11-acre field. Eight acres of this field received 20 pounds per acre of 10% phorate granules (2 pounds actual) applied in the same manner as described above. The remainder of this field was treated three times during the growing season with a dust mixture containing 2% parathion +50% sulfur. The entire field was divided into quadrants for sampling and counting aphid and psyllid populations at weekly intervals.

In this test and in Test No. 1 a heavy population of green peach aphids developed by the time the plants were 2" to 3" high. The first counts were made on April 12 (table 3) following the first irrigation and first application of sulfur dust. The aphid population in the phorate-treated plot was only 2 aphids per 25 leaves, while in the plot dusted with parathion-sulfur there were 139. The increase in aphid population in the phorate-treated plot from May 10 to May 24 may have been caused by maturity and senescence of the foliage or by a reduction in phorate metabolites as compared with it in the less mature leaves. The aphids were found on older, more mature leaves. These leaves apparently did not receive as much of the phorate-metabolites as they did during earlier rapid foliar growth. During the same period the population in the plot receiving parathion-sulfur dust increased to as high as 1,444 aphids per 25 leaves. The sudden decrease on June 1 occurred concurrently with existing warm weather and an application of insecticide.

A small population of potato psyllids in the plot dusted with parathion-sulfur was controlled with the first application. No psyllids were found in the phorate-treated plot during the entire season.

Results in table 3 show that the thrips populations of April 19 and 27 were much higher in the plot treated with parathion-sulfur than in the phorate-treated plots. A second dusting about May 3 cleaned up the thrips, but by the third week they were building up again. In the phorate-treated plot the thrips population remained low.

Total yield per acre from the plot treated with parathion-sulfur was 10.2% larger than the yield from the phorate-treated plot (table 4). This was partly caused by the different harvest dates. Phorate-treated potatoes were dug June 16 and the plots treated with parathion-sulfur were harvested on July 28. Harvesting dates were assigned by the grower to suit his convenience.

Table 2.—Red Pontiac potato yields from phorate-treated and untreated plots, Mesa, Arizona, 1960.

U. S. Grade	Number of 100-lb. Sacks per Acre			
	Untreated		Phorate-Treated	
	Sacks	Per Cent	Sacks	Per Cent
No. 1-A	86.5	45.1	230.6	67.9
No. 1-B	91.6	47.8	15.3	4.5
No. 1-Jumbo	0.6	0.3	75.3	22.2
No. 2	4.9	2.5	8.8	2.6
Culls	8.2	4.3	9.8	2.9
Total/acre	19,181 lb.		33,962 lb.	

Table 3.—Control of insects on Kennebec potatoes[a] with phorate. Queen Creek, Arizona, 1960.

Sampling Dates	Number of Insects per 25 Leaves					
	Parathion-Sulfur Treated			Phorate-Treated		
	Green Peach Aphids	Psyllids	Thrips	Green Peach Aphids	Psyllids	Thrips
4/12[b]	139	0.8	—	2.0	0	—
4/19	185	0	20.5	2.0	0	5.5
4/27	627	0	54.5	2.5	0	2.5
5/3[b]	248	0	0.0	0.4	0	0.0
5/10	739	0	0.0	23.3	0	0.0
5/18	1,444	0	7.0	312.6	0	1.3
5/24	1,297	0	5.0	931.8	0	0.5
6/1[b]	23	0	0.8	521.3	0	0.0

[a] Planted February 6.
[b] Indicates approximate dates of dust applications.

Table 4.—Kennebec potato yields from plots treated with phorate or parathion-sulfur. Queen Creek, Arizona, 1960.

U. S. Grade	Number of 100-lb. Sacks per Acre			
	Parathion-Sulfur Treated[a]		Phorate-Treated[b]	
	Sacks	Per Cent	Sacks	Per Cent
No. 1-A	188.7	45.1	187.5	49.93
No. 1-B	9.2	2.5	10.4	2.76
No. 1-Jumbo	156.9	37.5	151.9	40.45
No. 2	28.5	6.8	19.6	5.22
Culls	35.6	8.5	6.1	1.62
Total/acre	41,838 lb.		37,550 lb.	

[a] Harvested July 28.
[b] Harvested June 16.

Table 5.—Control of insects on Red Pontiac potatoes[a] treated with phorate granules or parathion-sulfur dust. Queen Creek, Arizona, 1960.

Sampling Dates	Number of Insects per 25 Leaves					
	Parathion-Sulfur Dust			Phorate Granules		
	Green Peach Aphids	Psyllids	Thrips	Green Peach Aphids	Psyllids	Thrips
4/13[b]	5	0.2	26	9	0	16.7
4/20	13	0	207	5	0	6.3
4/27	10	0	555	15	0	8.3
5/5	66	0	1	10	0.2	1.0
5/10	183	0	1	13	0	0.0
5/18	603	0	12	78	0.2	0.5
5/24	226	0	9	60	0	0.0
6/1[b]	4	0	1	6	0	0.0

[a] Planted and treated February 19.
[b] Indicates approximate dates dust was applied.

Differences in yield for U. S. grades 1-A, 1-B, and 1-Jumbo were not significant between treatments. A greater number of U. S. No. 2 and cull potatoes were harvested from the plot treated with parathion-sulfur.

TEST NO. 3—QUEEN CREEK, ARIZONA.—In the third test, also conducted in the Queen Creek area, the Red Pontiac variety was planted February 19 in a 10-acre field. Approximately 4½ acres of the field were dusted for adequate insect control with parathion-sulfur dust. The remainder of the field received 10% phorate granules at planting time, applied in the manner described above. Both plots were divided into quadrants and sampled for a total of 25 leaves per quadrant. Initial counts on April 13 indicated a low aphid population in this field. Data presented in table 5 show that the population increased in the dusted plot until May 18 when a maximum of 603 aphids per 25 leaves was found. In the phorate-treated plot the aphids increased to 78 per 25 leaves by the same date, then decreased as the plants matured.

A small initial population of psyllids was found in the dusted plots on May 13. In the phorate-treated plot a few isolated psyllids were found on May 5 and May 18. These

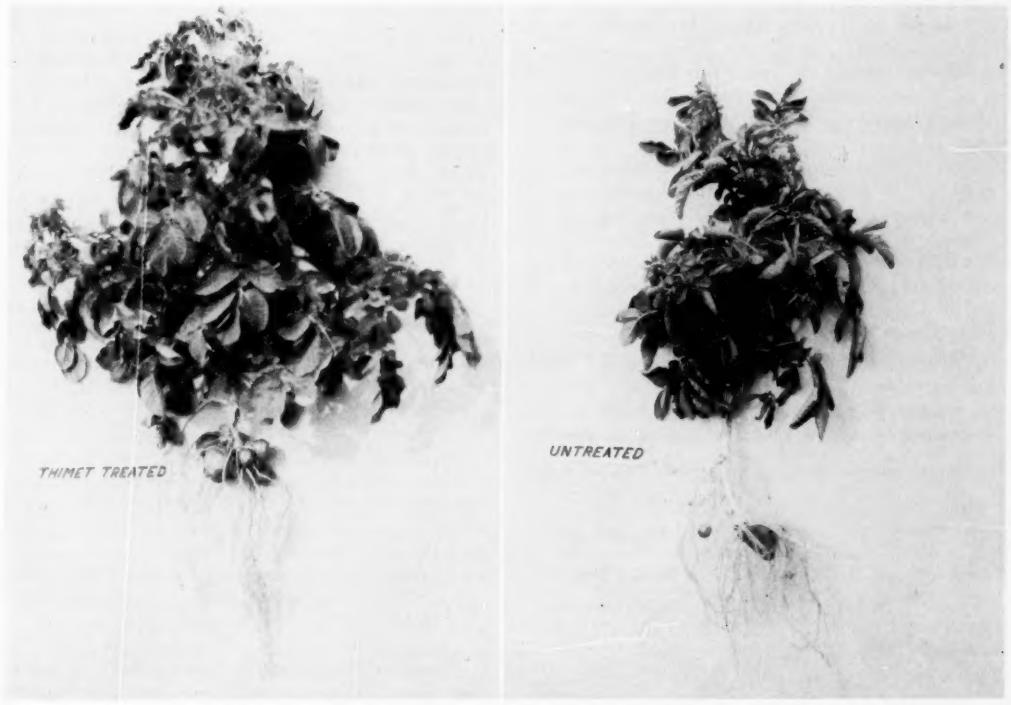

FIG. 1.—Early tuber set on phorate (Thimet)-treated and untreated Red Pontiac potato plants, Mesa (Arizona) Branch Station, 1960.

Table 6.—Control of insects with phorate granules on Red Pontiac potatoes.[a] Picacho, Arizona, 1960.

Sampling Dates	Number of Insects per 25 Leaves					
	Untreated			Phorate Treated		
	Green Peach Aphids	Psyllids	Thrips	Green Peach Aphids	Psyllids	Thrips
4/8	213	0	—	6.8	0	—
4/14	61	1.3	68.5	1.4	0	10.3
4/22	60	20.8	21.5	0.8	0	10.7
4/29	42	43.3	14.0	0.7	0	1.7
5/6	22	27.3	1.0	0.3	0	0.3
5/11	8	5.8	1.0	0.6	0	0.0
5/20	4	0.3	2.0	0.6	0	0.5
5/26	4	5.0	1.0	2.6	0	0.5
6/3	9	0	1.0	26.9	0	0.0

[a] Planted and treated February 19.

infestations did not increase and no symptoms of psyllid yellows were observed in this field. Although the control of aphids and psyllids was not so outstanding in this field, it is believed that the phorate treatment did reduce psyllid, aphid, and thrips populations and kept them at a low level. No data on yields were obtained from this field.

TEST NO. 4—PICACHO, ARIZONA.—This test was conducted near Picacho in the Casa Grande Valley where 11½ acres were planted on February 17 to Red Pontiac potatoes. A 10% phorate granulated insecticide was applied in one of the fertilizer bands as the seed pieces were planted. A one-quarter-acre untreated area was left on the east side of the field for use in ascertaining the existing insect population potential.

The field was divided into quadrants and a total of 50 leaves in each was examined for the presence of aphids, psyllids and thrips. Results of this test are presented in table 6.

The first count on April 8 shows a high aphid population of 213 per 25 leaves in the untreated plot compared with the lower count of 6.8 aphids in the phorate-treated plot. One week later, on April 14, the first psyllids were found in the untreated plot. This infestation continued to increase reaching a maximum of 43.3 psyllids per 25 leaves on April 29. Psyllids were still present as late as May 26, a few weeks before harvest. The majority of the plants in the untreated plot developed psyllid yellows, which became more severe as the season progressed. The tubers at this time were smaller and fewer than those in the treated plot. At no time during the growing season were any psyllids found in the phorate-treated plot.

The difference in thrips population was not so great as with the aphids and psyllids. Observations indicated considerably less thrips damage to the foliage in the treated than in the untreated plot.

As shown in table 7, the untreated plot produced 28% fewer potatoes per acre than the treated plot. The data also show a greater yield of the U. S. No. 1-A size per acre from the phorate-treated plot than from the untreated plot. Comparisons between yields of No. 1-Jumbo, 1-B's, and 2's are also shown in table 7. The increased yield of the more desirable sizes may probably be attributed to the control of the potato psyllids by the phorate.

TEST NO. 5—PICACHO, ARIZONA.—This experimental field in the Picacho area was planted to Red Pontiac potatoes on February 21. The field was treated with 1,000 pounds per acre of 0.2% phorate granular formulation (2 pounds actual) incorporated in a commercial 10-20-5 fertilizer mixture. The mixture was applied in bands on both sides of the seed row at planting time.

Data given in table 8 show that the field was free of psyllids during the entire growing period of 105 days. Likewise the population of green peach aphids was low, with 9.2 aphids per 25 leaves. All counts were made after the first irrigation on April 1. The aphid population continued to decrease until about 90 to 100 days after treatment when the population began to build up as in other phorate-treated fields in the area.

The thrips population was high during the early part of the growing season but dropped to zero by May 6. A slight increase in thrips population occurred just before harvest.

DISCUSSION.—Control of early infestations of the potato psyllid is of great importance in Arizona potato-growing areas. Failure to control this insect results in the development of psyllid yellows. As shown in Tests No. 1 and No. 4, yields from infected fields are decidedly reduced. From field tests conducted in Arizona during 1959 and 1960 it was shown that granulated phorate applied at

Table 7.—Red Pontiac potato yields from phorate-treated and untreated plots. Santa Cruz Farms, Picacho, Arizona, 1960.

U. S. Grade	Number of 100-lb. Sacks per Acre			
	Untreated		Phorate-Treated	
	Sacks	Per Cent	Sacks	Per Cent
No. 1-A	176	64.1	300	78.5
No. 1-B	83	30.1	46	12.0
No. 1-Jumbo	2	0.7	14	3.7
No. 2	12	4.4	18	4.7
Culls	2	0.7	4	1.1
Total/acre	27,500 lb.		38,200 lb.	

Table 8.—Results of phorate-fertilizer mixture applied at planting time for insect control on Red Pontiac potatoes.[a] Picacho, Arizona, 1960.

Sampling Dates	Number of Insects per 25 Leaves		
	Granulated Phorate-Fertilizer Mixture[b]		
	Green Peach Aphids	Potato Psyllids	Thrips
4/8	9.2	0	—
4/14	7.7	0	90.0
4/22	3.2	0	30.0
4/29	0.3	0	40.0
5/6	0.5	0	0.0
5/11	1.2	0	0.0
5/20	1.2	0	2.5
5/26	1.5	0	0.0
6/3	13.2	0	5.0

[a] Planted and treated February 19. First irrigation April 1.
[b] Mixture contained 0.2% phorate granules plus fertilizer equivalent of 10% nitrogen+20% phosphoric acid +5% potash.

planting time at the rate of 2 pounds actual phorate per acre gave season-long control of the potato psyllid and the green peach aphid. The effectiveness of this dosage confirms results of Bacon (1960) working in California with green peach aphids on potatoes.

The data presented indicate that the phorate granules placed in the fertilizer band on one side of the seed provide effective control. Effectiveness was not improved when it was applied in the fertilizer to both sides of the row.

It is important that at least one irrigation be made as soon as possible after the plants have emerged. This irrigation is necessary to put the phorate into the soil solution so that it can be absorbed by the roots and translocated to the aerial portion of the plant where the insects are feeding. In addition to controlling the potato psyllid and green peach aphid the phorate treatment also was effective in reducing populations of thrips and leafhoppers.

A slight buildup of aphids was observed in phorate-treated fields late in the season as the vines matured just before harvest. No reduction in yield was observed in any of the fields where phorate had been applied in the fertilizer band at planting time.

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The Jack-Pine Budworm as a Pest of Other Conifers with Special Reference to Red Pine.[1]

HERBERT M. KULMAN and ALEXANDER C. HODSON[2]

ABSTRACT

The jack-pine budworm, *Choristoneura pinus* Freeman, was observed feeding in an apparently normal manner on eastern larch. Observations in 1956 and 1957 indicated that large populations of the jack-pine budworm occurred on staminate-cone-producing red pine. The budworm defoliation caused top killing on red pine in all positions of the stand and it was shown that the dead portion of the crown was correlated with the length that was 80% or more defoliated. It was shown also that there was a correlation between the 1957 defoliation and the 1958 terminal growth on understory red pine. The 1957 terminal growth was not correlated with the 1957 defoliation.

The jack-pine budworm, *Choristoneura pinus* Freeman, has been found on several conifers other than jack pine. Although most of these records are considered incidental, there are a few cases in which the budworm has caused considerable damage.

Graham (1935) states that Scotch pine is favored over jack pine and that the preference order for pine is Scotch, jack, red, and white. Brown & MacKay (1943) reared jack-pine budworm from jack, Scotch, white, mugho, and lodgepole pine and from white and black spruce. Heron & Prentice (1957) report damage to planted jack, Scotch, and lodgepole pine by the jack-pine budworm. Freeman (1953) in the description of *Choristoneura pinus* as a separate species gives both jack and red pine as host trees. Reeks (1958) noted that Scotch and lodgepole pine were more seriously damaged by the jack-pine budworm than jack pine. Dead tops were found in 18 Scotch pine plantations in Manitoba. Prentice & Nairn (1958), working in the same plantations, found marked reductions in growth of Scotch pine in the year of defoliation and in the following year.

Hodson & Zehngraff (1946) state that ". . . reproduction of jack pine or other species such as white or red pine growing under heavily infested trees frequently is killed or injured severely in one season." Shenefelt & Benjamin (1955) reported that budworm larvae are blown onto white pine, white and Norway spruce, and balsam fir where they can feed and complete their life cycle and sometimes cause severe damage.

HOSTS AND DEFOLIATION.—In June and July of 1958 the jack-pine budworm was observed feeding on planted white spruce near Bemidji, Minnesota. During the same year it was observed feeding on plantations of white, red, Scotch and jack pine, white spruce, and eastern larch at Badoura, Minnesota. It is believed that this is the first record of the jack-pine budworm on eastern larch. It was actively feeding and webbing together partly eaten needles. The budworm was easily found on all the larch growing on the edge of the plantation. Nearby red, white, Scotch, and jack pines were much more heavily infested, but all populations were too light to cause any serious defoliation.

Jack-pine budworm defoliation damage to jack pine is usually concentrated in the tops of the trees, but entire jack pine trees are not infrequently stripped of needles

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[2] Assistant Professor of Entomology, West Virginia University and Professor of Entomology and Economic Zoology, University of Minnesota, respectively.

Table 1.—Jack-pine budworm damage to red pine growing in jack pine stands.

Height Class (feet)	Per Cent of Trees in Each Height Class								Total Trees Examined
	No Damage	Thin Foliage	Dead Tips	Length Distribution of Dead Tips to the Nearest Foot					
				1	2	3	4	5	
10–19	100	0	0	0	0	0	0	0	3
20–29	82	0	18	0	9	0	0	9	11
30–39	37	0	63	15	26	7	4	11	27
40–49	26	8	66	24	24	6	6	6	50
50–59	26	11	63	32	5	0	21	5	19
Over 59	0	25	75	0	0	0	0	0	4

and thus killed. In a mixed jack and red pine area north of Bemidji that was defoliated in 1954 and 1955, several flat-top overstory red pines were noticed to have dead tops in 1956. Some of these trees were about 20 feet taller than the surrounding jack pine trees. In the summer of 1957 severe top defoliation of all crown classes of red pine in jack pine stands was observed near Cloquet, Pequot Lakes, and in areas of Hubbard County south of Bemidji. See figures 1 and 2. In the Bemidji area the crowns of several trees were examined. Heavy populations of budworm were found along with a heavy crop of staminate cones. Staminate cones were detected in other areas with binocular field glasses. In all areas the surrounding jack pine stands were only lightly infested with the budworm and staminate cones were scarce. The association of the budworm with staminate cones on red pine was consistent with the findings of Graham (1935), Hodson & Zehngraff (1946), and Lejeune & Black (1950), who established the importance of staminate cones to the success of budworm populations of jack pine.

DEFOLIATION AND TOP KILLING.—In order to investigate the association of defoliation and top killing, 28 trees at the Pequot Lakes and Hubbard County areas were marked and an ocular estimate made of the defoliation in the topmost portion of the crowns. Both per cent defoliation and length of crown defoliated were recorded. In the fall of 1958 the trees were reexamined and an estimate made of the length of the crown that was dead. Trees that were 70% or less defoliated did not suffer any crown killing, but trees that were 80% or more defoliated suffered crown killing in direct proportion to the length defoliated. The data from the 22 trees that were 80% or more defoliated were used to calculate components for the normal equation $Y = mX + a$, according to Cox (1954), in which Y = length of dead crown observed in 1958 and X = the length of crown defoliated 80% or more in 1957. The m and a components were 0.581 and 1.043, respectively. The correlation coefficient r was 0.558, which was significant at the 5% level—the required correlation coefficients for the 1% and 5% levels of significance are 0.561 and 0.444, respectively. From the foregoing data it was concluded that 80% or more defoliation of the tops of crowns of red pine results in a directly proportional length of top killing. In addition to this damage, isolated dead branch tips were found on many trees.

In almost all areas examined, damage was also observed on red pine reproduction. At Pequot Lakes and Solway 21 red pine trees under 12 feet tall suffering varying degrees of defoliation were marked in 1957 and reexamined in 1958. Here, as with overstory red pine, top mortality followed defoliation of 80% or more of the needles. While making the 1958 observations on some of the reproduction sized trees, it was noticed that the 1958 terminal growth was very much affected by the defoliation history. In order to make a measurement of this effect, the 1954 through 1958 terminal growth was measured on 35 understory trees at Pequot Lakes and on 25 understory trees at Solway that suffered varying degrees of defoliation in 1957. The terminal growth for 1957 and 1958 was calculated as a per cent of the 1954 through 1956 terminal growth in order to compensate for individual tree differences. The per cents were then subjected to an angular transformation (arc sin). The normal equation $Y = mX + a$ was calculated in which Y = terminal growth in inches (transformed) and X = per cent of defoliation on the 1954 to 1957 terminal growth. At both locations the 1957 calculations were not significant thus suggesting that the 1957 defoliation did not affect the 1957 terminal growth. This was expected because the defoliation usually does not occur until most of the terminal growth has occurred. The 1958 growth, however, was correlated with the 1957 defoliation. The m and a values for Pequot Lakes are -7.3647 and 40.4846 and the respective values for Solway are -10.2737 and 44.0713. The respective correlation coefficients r are 0.668 and 0.825—the required coefficients for significance at the 1% level are 0.449 and 0.526, respectively. These findings are particularly interesting relative to the reports of Büsgen & Münch (1929) in which they stated that radial growth is much more likely to be influenced by current weather conditions than shoot growth, which is determined almost entirely by the quantity of available materials stored during the previous season.

QUANTITY OF DAMAGE.—In order to investigate the quantity of damage that occurs on red pine in jack pine stands, two areas in Hubbard County, each about 3 acres in size, were examined in 1958 and records taken as to the condition of the red pine. The red pine was defoliated in 1957 and as in the other Hubbard County areas only very light defoliation occurred on the associated jack pine. These data are presented in table 1. The trees ranged in size from old overstory wolf trees to progressive,[3] provisional,[3] and regressive[3] trees physically associated with the jack pine. It appears that the per cent of trees with

[3] Head dominants; conditional and codominants; and weak dominants, codominants, and intermediates, respectively, as interpreted from Gevorkiantz et al. (1943)

Table 2.—Damage to understory red pine in jack pine stands caused by jack-pine budworm defoliation.

Height Class (feet)	Per Cent of Trees					Total Trees Examined
	No Damage	Tip Dead	1 Foot Dead	2–5 Feet Dead	5–10 Feet Dead	
3–10	44	24	20	12	0	59
11–20	72	5	9	14	0	93
21–30	31	3	10	35	21	29
31–40	37	0	9	27	27	11
41–50	100	0	0	0	0	3

FIGURES 1 and 2.—Dead tops on red pines caused by jack-pine budworm defoliation.

dead tops increased with the height class of the trees. This was probably associated with the fact that staminate cone production is usually heavier on the larger trees and that the budworm tends to oviposit on the tops of overstory trees.

In an area near Pequot Lakes a 55- to 65-foot tall, 55- to 60-year old, closed, jack pine stand was heavily defoliated in 1956. Understory pole and reproduction sized red pine were defoliated by budworms that probably originated on the jack pine overstory. A survey of the damage to red pine was conducted in 1957. These data are presented in table 2. More than 40% of the trees had suffered some degree of top mortality. The absence of an association between the amount of damage and height of trees in these data is probably because the trees were in the understory and defoliated by budworms that originated in the jack pine overstory.

DISCUSSION.—The foregoing studies illustrate that the jack-pine budworm can cause serious damage to red pine whether it is towering over jack pine, associated in the canopy with jack pine, or understory trees in a jack pine stand. The incidence of red pine defoliation in 1957 when the associated jack pine was not defoliated strongly suggests that the jack-pine budworm can survive independently on red pine. Jack pine, but not red pine, was defoliated in 1956. The survival of the insect on either host is associated with staminate cones as pointed out earlier. It would have been necessary, however, that budworm eggs be present on the correct host. Therefore, the budworm must have deposited enough eggs on red pine to account for the defoliation at least for the red pine that towered over the jack pine. Since jack and red pine do on occasions produce staminate cones in alternate years, it appears that red pine may serve as a carryover host for the jack-pine budworm and, therefore, may help to maintain the budworm at a subepidemic level between periods of staminate cone production on jack pine.

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Fecundity and Longevity of the Adult Female Pink Bollworm Reared on Natural and Synthetic Diets[1]

PERRY L. ADKISSON,[2] *Texas Agricultural Experiment Station, College Station*

ABSTRACT

Oviposition and longevity records were taken of adult female pink bollworms, *Pectinophora gossypiella* (Saunders), reared from cotton squares, green bolls, 1% and 5% cottonseed meal diets and a wheat germ diet. The insects were paired and maintained in individual cages at 80° F. Average egg production per female from the various larval rations was: square-reared moths, 98.1 eggs; boll-reared, 204.3; 1% meal, 336.7; 5% meal, 302.1; and wheat germ, 312.2. The average duration of the adult life span for females reared from the various diets ranged from from 15.4 to 17.2 days. Highly significant correlations were found between body weight and egg production and longevity and egg production. Generally, the heaviest females and those that lived the longest periods produced the greatest number of eggs. There was no correlation between body weight and longevity.

The pink bollworm, *Pectinophora gossypiella* (Saunders), is primarily a late-season pest of cotton. Studies of the seasonal history of the insect indicate, in general, that infestations are usually very light during the early fruiting period of cotton and may not become noticeable until the plants attain the bloom stage. After this period has been attained and as bolls become available for oviposition and a source of larval food, the infestation may increase rapidly. All the bolls may become infested with one or more larvae by the end of the season. However, severity of the attack will vary from field to field and from season

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[2] Associate Professor, Department of Entomology. The author gratefully acknowledges the assistance of Mrs. Louise Lewis, Laboratory Assistant, for her help in the conduct of this study.

to season (Loftin et al. 1921, Ohlendorf 1926, Taylor 1936, Pearson 1958).

Several reasons for this characteristic rate of seasonal population increase have been expressed. Loftin et al. (1921) believed that one reason for the gradual increase in infestation was caused by the extremely small numbers of pink bollworms that are able to survive the winter. Pearson (1958) stated that the rate of multiplication in the crop appears to be governed primarily by the temperature. Adkisson & Gaines (1960) suggested that the biotic potential of the insect during the pre-boll stage of the host plant was limited because of reduced fecundity of the females and the high natural mortality of young larvae during this stage of plant growth. Probably, all these factors contribute to lighter infestations before the boll period. Since the rapid increase in infestation appears to coincide with the appearance of blooms and bolls, there is a possibility that the source of the larval diet and the availability of nectar for the adults to feed on are also important factors governing the rate of population increase.

Willcocks (1916) and Taylor (1936) reported that large pink bollworm females may produce 350 to 500 eggs while small moths may produce only 200 to 250. Fenton & Owen (1953) found that moths reared from green cotton bolls produced more eggs and lived longer than those collected from squares. Lukefahr & Griffin (1956) found that adults fed on a honey solution or a simulated cotton nectar laid 2 to 2½ times as many eggs as those furnished water only. Brazzel & Martin (1957) reported that the reproductive capacity of moths caged on cotton plants at regular intervals increased as the season progressed even though all the moths were obtained from diapause larvae collected from the same source. These reports indicate that nutrition during larval and adult stages of the insect through the season could have an important bearing on the rate of population increase.

PROCEDURE.—Pink bollworm moths reared from several larval sources were paired in individual cages on the day of emergence from the pupal stage. Larval sources included cotton squares, green cotton bolls, two formulations of the cottonseed meal diet and a wheat germ diet.

Square-reared adults were collected on emergence from cotton squares, which had been picked from infested plants, and held in special emergence cages. Squares were picked at random from infested plants maintained in large plastic screen cages. After collection, the squares were spread in a thin layer on an insectary bench and allowed to dry for 2 to 3 days. After drying, they were placed in a small screened cage. The squares were moistened at irregular intervals during the holding period to prevent excessive drying. The exterior surface of the emergence cage was covered with heavy butcher's-paper and fitted with an 8-dram shell vial which served as a collection tube. As the moths emerged, they would congregate in the tube. The moths were removed daily.

Fresh green cotton bolls were also collected from the above-mentioned plants. These bolls were placed in 1-gallon cans which had the tops and bottoms removed. A circular piece of hardware cloth of appropriate size was fitted into the bottom of each can to support the bolls. The cans were placed over a wad of cotton. The larvae on emergence from the bolls would enter the cotton and spin a cocoon for pupation. The pupae were removed from the cotton and confined in individual gelatin capsules until transformation to adult was completed.

Larvae were reared on two formulations of a cottonseed meal diet and on a wheat germ diet according to the methods outlined by Bull & Adkisson (1960) and Adkisson et al. (1960). The cottonseed meal diets, hereafter designated in this paper as the 1%- and 5%-meal diets, were prepared by solvent extraction of raw cottonseed meal with n-hexane to an oil content of less than 1% of the total dry weight. Wesson oil was added to the finished diets in the amounts of 1% or 5% (w/w) depending on the diet desired. Other ingredients in the diets are given in the above references. The total fat content of the laboratory diets as determined by ether extraction on a dry weight basis were 1% meal, 1.6% fat; 5% meal, 5.6% fat and wheat germ 0.3% fat. The insects were held in individual rearing containers until they completed development to the adult stage.

Newly emerged adults were collected daily, weighed, sexed and paired. Paired females and males were always collected from the same larval source. The pairs were maintained separately in individual oviposition cages. The oviposition cages were constructed from 1-pint ice cream cartons. A circular opening was cut in the top of each cage. A piece of 14×18 plastic screen was glued over this opening. Each day a fresh cotton leaf was placed over this screened opening and held in place with a dampened paper towel and a small weight. The female moths would oviposit on the screen, on the cotton leaves and on the paper towels. The insides of the cartons were coated with paraffin to discourage any oviposition on the internal surfaces of the cages since it has been found that females seldom oviposit on smooth surfaces. Each cage was also fitted with a small cotton-stoppered vial of a sugar-water solution which served as food for the adults.

The cotton leaves, the plastic screen and the paper towels were examined daily for eggs. Examinations were made with the aid of a binocular microscope. Oviposition records were made at the end of the first 24-hour period after caging and were continued until the death of the female. Records were maintained only from females that produced one or more eggs. Adults were held in a darkened room at a constant temperature of 80° F. ±2°.

Data collected during this study were analyzed by appropriate statistical methods by the Data Processing Center of the Texas A & M College System.

RESULTS.—Fecundity and longevity records of the females collected from the various larval diets are given in table 1. The females collected from natural food sources (squares and bolls) produced fewer eggs than those reared from synthetic diets. The square-reared females laid the fewest eggs and the moths collected from the 1%-meal diet the largest number. The boll-reared females produced approximately twice as many eggs as the females reared from squares. The statistical analysis indicated significant differences in egg production between the square-reared and boll-reared females, between square-reared and diet-reared moths and between boll-reared and diet-reared individuals. There were no significant differences in the number of eggs produced by females reared from the various synthetic diets. The smallest number of eggs produced by any individual was 1 and the largest was 801.

The preoviposition period for all individuals averaged approximately 4 days. The square-reared individuals

Table 1.—Fecundity and longevity records of female pink bollworm moths collected from various larval sources.

Larval Source	No. of Females Observed[a]	Average Adult Weight (mg.)	Egg Production: Average per Female	Egg Production: Range	Egg Production: Average per Moth Day	Days: Average Preoviposition Period	Days: Range	Days: Average Adult Longevity	Days: Range
Squares	38	4.2	98.1	2–431	6.3	5.4	1–13	15.5	8–25
Bolls	32	13.2	204.3	3–790	13.2	4.5	1–11	15.4	7–35
5% meal	45	14.9	302.1	2–634	19.0	3.9	1–11	15.9	7–32
1% meal	55	16.2	336.7	2–792	20.9	4.2	1–13	16.1	5–29
Wheatgerm	61	15.1	312.2	1–801	18.2	3.8	1–15	17.2	7–30

[a] Records taken only from females that produced at least one egg. Means enclosed in common side-brackets are equal and are significantly different from other means in the array at the 1% level of probability.

required the longest preoviposition period, 5.4 days, and the wheat-germ-media-reared females the shortest, 3.8 days. The preoviposition period ranged from 1 to 15 days.

There were no significant differences in the duration of adult longevity among the females reared from all larval sources. The average period of the adult life was approximately 16 days and ranged from 5 to 35 days.

The number of days of adult life was divided into the number of eggs laid per female to obtain the rate of egg production per moth day. The females reared from squares produced considerably fewer eggs per day of life than moths reared from the other sources.

There appeared to be a relationship between adult longevity and the number of eggs produced. A correlation analysis substantiated this as the computed r value was significant at the 1% level of probability. The data indicated that in general the females that lived the longest produced the greatest number of eggs.

Apparently there was no relationship between body weight and length of the adult life since the computed r value was not significant. The light-weight moths lived approximately as long as the heavier individuals.

Weight records showed that the adult females reared from green bolls and synthetic diets were approximately 3 to 4 times heavier than the square-reared individuals. Since there appeared to be a relationship between body weight and total eggs produced, a correlation analysis of the available data was made. Analyses were made on the basis of both pupal and adult weights. The r values calculated for both pupae and adults indicated a highly significant correlation between body weight and the number of eggs produced. The largest females consistently produced more eggs than the smaller individuals.

The method used for collecting square-reared moths in the present study could possibly have resulted in smaller moths than would be found in the field from similar sources. The larvae in this study developed from dead and decaying squares. Though some larvae in the field may develop from similar tissue in infested shed squares (Taylor 1936), it is generally thought that most of the square-reared pink bollworms develop in living or succulent squares that remain on the plant. Since no reports of the weights of larvae collected from various sources could be found in the literature, nondiapause square-reared larvae were collected from several sources and weighed on a Roller-Smith balance. Weights were made only of full-size fourth-instar larvae. The number of insects weighed, larval source and average weight per insect were: 61 larvae from cotton blooms averaged 17.0 mg. each; 165 larvae from green bolls averaged 24.0 mg. each; 80 larvae each from wheat germ, 5% and 1% meal diets averaged, respectively, 29.7, 28.0 and 29.9 mg. per individual. Since the correlation analysis indicated a significant correlation between body weight and reproductive capacity, it appears likely that the average square-reared female will produce fewer eggs than one reared from a boll. This is in agreement with previous findings reported by Taylor (1936) and Fenton & Owen (1953).

Table 2.—Average egg production of females that laid more than 16 eggs.[a]

Larval Ration	Number Females	Average No. Eggs/ Female	Average Longevity (days)	Average No. Eggs per Day
Squares	31	119.0	15.7	7.6
Bolls	31	210.7	15.5	13.6
5% meal	42	323.5	16.2	20.0
1% meal	49	376.9	16.3	23.2
Wheatgerm	56	393.3	17.3	19.6

[a] Means enclosed in common side-brackets are equal and are significantly different from other means in the array at the 1% level of probability.

Since there was a possibility that the above results may have been biased by differences in the percentages of females that mated among the various treatments, a comparison was made only from individuals that produced 16 or more eggs. This assumption was based on observations made at this laboratory that unmated individuals seldom lay more than 10 to 15 eggs. The results, as shown in table 2, indicated little difference in the relative productivity or longevity of these females from that presented in table 1.

The daily and weekly rate of egg production by the females is presented in table 3. Considering all females under observation, the greatest number of eggs were produced on the seventh night following caging. Of the total number of eggs produced by all females, 50.5% were laid within the first week of life and 43.3% were produced during the second week. Thus, 93.8% of all eggs produced were laid during the first 14 days of the adult life.

DISCUSSION.—That the quality of the larval diet has an important function in determining the reproductive capacity and the longevity of the female pink bollworm moth is not unexpected. Wellso & Adkisson (1961) found that the ovaries of females reared from the wheat germ diet may contain 550 or more eggs in various stages of development at the time the moth emerges from the pupal case. This number greatly exceeds the number of eggs that is generally deposited by an average female pink bollworm moth. It appears from the standpoint of fecun-

Table 3.—Rate of egg production by female pink bollworms collected from several larval sources.

DAYS OF ADULT LIFE	LARVAL RATION					DAILY TOTALS	WEEKLY TOTALS	WEEKLY % OF TOTAL
	Squares	Bolls	5% Meal	1% Meal	Wheat Germ			
1	16	10	0	3	0	29		
2	121	110	113	399	445	1,188		
3	322	66	574	1,148	731	2,841		
4	289	390	705	1,532	1,105	4,021		
5	304	761	2,231	2,356	1,595	7,247		
6	315	947	1,658	1,858	2,359	7,137		
7	797	828	1,537	2,130	3,253	8,545	31,008	50.5
8	383	796	1,097	1,898	1,701	5,875		
9	254	588	1,152	1,453	1,327	4,774		
10	364	517	1,335	1,395	1,362	4,973		
11	134	222	772	975	1,270	3,373		
12	101	376	825	800	862	2,964		
13	123	254	486	674	882	2,419		
14	141	174	514	551	814	2,194	26,572	43.3
15	30	88	223	295	287	923		
16	26	76	27	274	347	750		
17	3	45	126	821	291	746		
18	4	8	40	116	310	478		
19	2	231	69	150	43	495		
20		49	81	116	14	260		
21			13	48	8	69	3,721	6.0
22			6	13	0	19		
23			3	18	35	56		
24			0	19		19		
25			9	9		18		
26				0				
27				0				
28				6		6		
29				2		2	120	0.2
Total	3,729	6,536	13,596	18,519	19,041	61,421		

dity and longevity of the females that squares are inferior to bolls and bolls are inferior to the synthetic diets as larval food sources. The effect of larval food on the reproductive capacity of the adult and on the size of the insect are apparently related factors. Large females generally produce more eggs than small ones. This suggests that the reproductive capacity of female populations from any given larval source is proportional to the average size of the individual produced.

The findings of the present study combined with results in the literature reviewed furnishes some indication as to why pink bollworm populations generally increase rather gradually until fairly late in the growing season of the host plant. The pink bollworm apparently is not able to achieve maximum reproductive capacity until two conditions are fulfilled. These are (1) the female moths must have fed in bolls during the larval stage and (2) the adults must have a food source (Lukefahr & Griffin 1956). It is not until the third generation of this species that these requirements are met since prior to this period either the larvae are mostly square-reared or there is no floral nectar available to adults. As has been demonstrated, either of these factors can affect the reproductive capacity of the pink bollworm.

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Absorption and Translocation of Di-Syston by Cotton Plants[1]

CHING H. TSAO[2] and EDGAR W. CLARK,
Entomology Research Division, Agric. Res. Serv., U.S.D.A., Brownsville, Texas

ABSTRACT

The absorption and translocation of P^{32}-labeled Di-Syston® (*O,O*-diethyl *S*-[2-(ethylthio)ethyl] phosphorodithioate) were studied by means of foliar, seed, and soil treatments. The compound was readily translocated to leaves and stems from treated leaves or roots, but in these limited experiments it did not concentrate in fruiting forms. Distribution of Di-Syston leached from germinated and ungerminated treated cottonseed into the surrounding soil showed definite downward and, to a lesser degree, lateral movement.

Di-Syston® (*O,O*-diethyl *S*-[2-(ethylthio)ethyl] phosphorodithioate) and several other systemic insecticides, when applied as seed treatment or side dressing, gave control of cotton thrips and aphids for 3 to 6 weeks after the planting date (Parencia et al. 1957, Hanna 1958, Hopkins et al. 1958, Tuttle & Wene 1959). These insecticides applied to seed of cotton and two other crops were found in true leaves for at least 59 days after the treatment (Reynolds et al. 1957). The absorption and translocation of P^{32}-labeled Di-Syston by cotton plants following foliage, seed, and soil treatments are reported in this article.

MATERIALS AND METHODS.—The P^{32}-labeled Di-Syston[3] had an initial radioactivity of 193 counts per minute per microgram. Radioactivity was determined by means of a scaler with an end-window Geiger-Müller tube having a window thickness of 3.5 mg./cm.^{2}

Chloroform was used to extract the insecticide from plant materials and soil samples. Plant materials were first coarsely chopped and then homogenized in a glass blender for 20 minutes with chloroform, usually at a ratio of 40 ml. of the solvent to 10 g. of plant materials. Seeds that were treated with the insecticide were stripped with four 10-ml. portions of the solvent. Soil samples averaging 67.2 g. were processed by adding 100 ml. of chloroform to each in a quart jar, which was then rotated from end to end for 1 hour. In all preparations, the extracts were filtered through absorbent cotton and the volume of the extract measured to determine the amount recovered. All chloroform extracts were concentrated to 4 or 6 ml. at 40° C. under partial vacuum. Several 1- or 2-ml. aliquots of each concentrate were evaporated in planchets under an infrared lamp and then radioassayed. Specific methods will be found below.

RESULTS.—*Foliage Treatment.*—Five leaves on lower portions of each of five potted plants were treated by dipping in an emulsion containing 3.62 mg. of Di-Syston per ml. These leaves on each plant received an average of 3.3 ml. of the emulsion, or 12 mg. of the insecticide. Radioassay of treated leaves, and untreated leaves and stems of two plants collected 2 days after the treatment, gave the results shown in table 1.

Seed Treatment.—One gram of P^{32}-labeled Di-Syston was dissolved in a small amount of acetone and added to 1 gram of activated carbon. This mixture was stirred continuously until the acetone was completely evaporated. The impregnated carbon was used to treat 33.3 g. of mechanically delinted cotton seeds (Empire variety) by thoroughly mixing the two materials together. Since a portion of the impregnated carbon was not picked up by the seeds, the amount of insecticide that adhered to each seed was determined. The amounts of insecticide found in two lots were 7.67 and 8.17 mg., with an average of 7.94 mg. per seed. In the fourth stripping of the seeds, an average of 0.11% of the total amount of the insecticide was found.

Table 1.—Residues in different parts of cotton plants treated in different ways with P^{32}-labeled Di-Syston.

PLANT		RESIDUE	
Part Analyzed	Fresh Weight (g.)	μg.	p.p.m.
1. *Two days after foliage treatment (12 mg./leaf)*			
Treated leaves	26.7	948.5	35.5
Untreated leaves and stems	83.0	29.6	0.4
Untreated squares	5.0	0.4	.1
2. *Thirty-five days after planting treated seeds (7.49 mg./seed)*			
Roots	6.2	442.8	71.4
Stems	20.7	7.4	0.4
Leaves	41.2	165.4	4.0
Stems and leaves	61.9	172.8	2.8
Whole plant	68.1	615.6	9.1
3. *Two days after soil treatment (11 mg./plant)*			
Leaves and stems	104.6	56.7	0.5
Squares	12.3	0.4	.03
Firm bolls	84.1	0	0
Seeds of two firm bolls[a]	12.2	0	0

[a] Collected 21 days after treatment.

In the latter part of June about 50 treated seeds were planted in one row in the field. Three or four seeds were planted per hill, and the hills were 1 foot apart. Cages measuring 3×4×6 feet were placed over the row to protect the plants and comply with pink bollworm control regulations in this area that require the confinement of fruiting cotton plants under a screened cage after August 31. Three plants grown from the same hill were removed 35 days after planting. Extracts were made from leaves, stems, and roots of these plants. The amounts of the insecticide found in various parts of the plants are given in table 1. Because of late planting and heavy, late-season rainfall, all green bolls on the plants grown from treated seeds were damaged by rot; thus seeds from these plants were not available for residue determination.

Soil samples were taken at four depths with a soil auger ($\frac{3}{4}$-inch inside diameter) in the hill and 4 inches on either side of the hill where the three plants were removed. Soil samples were also collected at an adjacent hill where four damaged, ungerminated seeds were found. A total of 366 μg. of Di-Syston was found from two seeds on the soil

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[2] Now with the Department of Entomology, University of Georgia, Athens.
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Table 2.—P^{32}-labeled Di-Syston residue in soil adjacent to treated seeds (35 days after planting).

Depth of Soil (inches)	Beneath Plants		4 Inches on Either Side of Plants		Beneath Four Ungerminated Seeds	
	μg.	p.p.m.	μg.	p.p.m.	μg.	p.p.m.
0 – 5.9	229.3	5.4	44.6	0.28	156.9	2.35
6.0– 8.9	8.2	0.22	2.8	.02	133.5	2.16
9.0–11.4	14.3	.24	1.3	.01	48.1	0.82
11.5–13.5	15.8	.23	1.0	.01	33.3	.54
Amount recovered from soil	267.6	1.52	49.7	.08	371.8	1.44

surface, and 607 μg. from the other two seeds which were buried under 1 inch of soil. These amounts represent 2.3% to 3.8% of the original chemical deposits. Residual amounts of the insecticide found in these samples are given in table 2.

Soil Treatment.—Cotton plants grown in 1-gallon cans were treated by placing 3 ml. of an emulsion containing 11 mg. of the Di-Syston on the soil in each can, followed by the addition of 100 ml. of water. Pans placed under the cans caught any excess tracer-contaminated water draining from them. All plants bore squares and young bolls; some had firm bolls at the time of treatment. Two days after treatment leaves, stems, squares, and firm bolls of two plants were collected. Seeds from firm bolls on two other plants were collected 21 days after the treatment and extracted. Results of radioassay of these materials are given in table 1.

DISCUSSION.—The high concentration of the Di-Syston in the roots and the lower residues in the clay soil beneath the plants, compared with that beneath the ungerminated seeds, indicated that the insecticide was absorbed by the plants through roots (tables 1 and 2). The insecticide was translocated readily to leaves and stems from treated leaves or roots, but in these limited experiments it did not concentrate in fruiting forms on the treated plants (table 1).

Results shown in table 2 indicate that there was definite downward and, to a lesser degree, lateral movement of the Di-Syston in the clay soil from the treated seeds.

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Effects of Dietary Guthion on Baby Chicks[1]

PETER C. STEVE,[2] DONALD L. ANDERSON,[3] and J. H. LILLY[2,4]

ABSTRACT

Guthion® (*O,O*-dimethyl *S*-(4-oxo-1,2,3-benzotriazin-3-(4H)-ylmethyl) phosphorodithioate) was incorporated into chick starter feed at levels ranging from 9.5 p.p.m. to 2,177 p.p.m. and fed to day-old chicks. Within a week depression in gains in weight was observed at the 306-p.p.m. level, and depressions in gains in weight became progressively more severe as the concentration of Guthion was increased. At the 2,177-p.p.m. level 95% mortality occurred during the second week. Tests with starter feeds containing Guthion at levels of 100 and 600 p.p.m., alone and in combination with two different coccidiostats (Nicarbazin and Sulfaquinoxiline), did not reveal any adverse interactions. Chemical analyses of Guthion-feed mixtures, stored in metal containers with tight fitting lids, showed that Guthion disappeared fairly rapidly.

The possibilities of harmful effects of insecticide residues has stimulated considerable interest among various groups. There is need for more information on the effects of residues on the broad spectrum of animal life that eventually may be affected. Residue problems may also be complicated by antagonistic or other interactions resulting from the combination of two or more chemicals at low concentrations.

New chemicals for use on forage crops were being reviewed when this study was initiated. Guthion® (*O,O*-dimethyl *S*-(4-oxo-1,2,3-benzotriazin-3-(4H)-ylmethyl) phosphorodithioate) was one of the insecticides which appeared to be potentially useful. The possibility that Guthion residues on alfalfa might eventually find their way into commercial feeds via alfalfa meal formed the basis of this investigation.

The objectives of this study were three-fold. First, what levels of Guthion in starter feed are necessary to produce toxic symptoms in baby chicks? Second, is it practical to utilize newly hatched chicks as bioassay organisms for Guthion? Third, is there any interaction between Guthion and commercial coccidiostats when they are combined in the feed of young chicks?

METHODS AND PROCEDURES.—The New England College Conference chick starter feed without a coccidiostat served as the basal diet. Guthion in the form of a 25% wettable powder was added to the basal diet in weighed amounts to obtain the desired concentrations of insecticide. These mixtures were tumbled mechanically for at least 2 hours in 1-gallon glass containers, each of which held 4 pounds of the mixed feed. These jars also served as

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[2] Department of Entomology and Plant Pathology.

[3] Department of Poultry Science.

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Table 1.—Cumulative effects of graduated levels of Guthion in starter feed on day-old, single-combed, White Leghorn chicks of mixed sexes over a 4-week period.

Guthion in Feed (p.p.m.)	Initial Mean Weight (g.)	Cumulative Mean Weight Gains (gm.)[a]				Total Mortality (%)
		1 Week	2 Weeks	3 Weeks	4 Weeks	
Control[b]	40.6	27.7	82.3	142.2	211.1	7.5
Control[c]	39.4	25.9	74.1	132.6	195.6	2.5
74.8	39.2	23.3	76.9	139.2	215.5	10.0
149.7	39.0	25.6	74.0	132.9	203.9	5.0
299.4	38.9	21.3	64.9	126.9	192.6	0.0
598.4	39.6	11.6	50.2	86.4	149.8	15.0
1197.3	41.0	5.4	30.6	68.7	116.1	15.0
2177.0	39.5	1.5	—	—	—	95.0

[a] 20 chicks/treatment at start of test.
[b] "Tumbled" control—40 chicks/treatment.
[c] Regular control—40 chicks/treatment.

storage containers after mixing. Depression in gains in weight of the chicks was used as the criterion of Guthion toxicity.

In Experiment 1, each treatment consisted of two replicates, each containing 10 day-old, single-comb, White Leghorn chicks of mixed sexes. The chicks were identified by numbered wing bands and randomly assigned to pens in electrically heated battery brooders with raised wire floors. Feed and water were supplied *ad libitum*. Insecticide concentrations in the feed ranged from 74 p.p.m. to 2,177 p.p.m. to determine lethal, toxic and subtoxic levels. To check on possible effects of the mechanical mixing process, both tumbled and untumbled samples of the basal diet with insecticide were employed as controls. All birds were weighed individually at weekly intervals and records on feed consumption were kept throughout the course of the test. The results are summarized in table 1.

Experiment 2 was designed to include several levels of Guthion below 100 p.p.m., in an attempt to determine the effects of Guthion at concentrations which might approximate those levels of contamination which could result from actual use. Shaw & Smith (1960) reported that Guthion residues did not exceed 80 p.p.m. when this insecticide was applied to rapidly growing alfalfa in the field at one-half pound of wettable powder per acre. Two levels which caused depressions in weight in Experiment 1 were also included for comparison with the earlier results.

The procedures described for Experiment 1 were modified as follows for Experiment 2. (1) Feed containing weighed amounts of insecticide was mixed in a cement mixer in 20-pound lots and stored in 4-gallon metal containers with tight fitting lids. (2) Each replicate contained 20 chicks instead of 10. (3) A "carrier control," consisting of the inert ingredients used in the commercial Guthion formulation, was included to determine whether or not these ingredients were in part responsible for the lower gains in weight.

As in Experiment 1, the birds were identified by numbered wing bands and the treatments assigned randomly to the pens. Individual weights were recorded weekly for a 2-week period, since it had been established in Experiment 1 that the desired information could be obtained during this period. Feed consumption records were kept as in Experiment 1. These results are presented in table 2.

At the end of Experiment 2 samples of the feed mixtures were analyzed by the colorimetric method suggested by Adams (1959) to determine the amounts of Guthion present. These analyses were made by Professor C. Tyson Smith of the Control Service Laboratory of the University of Massachusetts. The results are shown in table 4.

Experiment 3 was designed to check further on a preliminary study in which there was an indication that birds receiving 74 p.p.m. of Guthion in a commercial starter feed containing an anti-coccidial drug gained more weight than control birds receiving only the commercial starter feed. Since Anderson (1959) and Lewis et al. (1960) showed that slight depression in gain in weight may result when a coccidiostat is included in feed at recommended levels (Sulfaquinoxiline, 0.0175% and Nicarbazin, 0.0125%), and that weight depressions were clearly evident above these levels, our data suggested a possible beneficial interaction between the coccidiostatic drug and this level of Guthion, particularly with male chicks.

In order to obtain maximum sensitivity in Experiment 3, only day-old males of a rapidly growing strain of broiler chicks were used. A 3×3 factorial design was employed to include toxic and sub-toxic levels of Guthion, alone and in combination with two coccidiostatic drugs, each at a dosage commonly found in commercial feeds. Fifteen chicks were assigned to each pen in brooder batteries as previously described. To avoid misplacement of chicks that might escape, each group was identified by a coding system in which the webbing between the toes was notched. Treatments were randomly distributed within the batteries and there were two replicates of each treatment. Feed preparation was carried out in the same manner as for Experiment 1. The chicks were group-weighed initially and at 1- and 2-week intervals. These results are shown in table 3.

RESULTS AND DISCUSSION.—Experiment 1 revealed that noticeable depression of gain in weight occurred approximately at the 300-p.p.m. level, but not at any of the lower levels employed. Table 1 shows that this depression was evident at the first weekly weighing. Early in the second week 95% mortality occurred at the 2,177 p.p.m. level, although the mortalities of chicks fed on lower Guthion levels were not in excess of that recorded for the control chicks. Although the chronic LC_{50} level was not determined, it was evident that it fell between the 1,197 p.p.m. and 2,177 p.p.m. dosages under the conditions of this experiment.

Feed consumption records showed that above the 300

Table 2.—Cumulative effects of relatively low levels of Guthion in starter feed on day-old White Leghorn chicks of mixed sexes, over a 2-week period.

Guthion in Feed (p.p.m.)	Initial Mean Weight (g.)[a]	End of 1st Week		End of 2nd Week		No. Dead at End of Test
		Mean Weight gain/ Chick (g.)	Avg. Feed Consumed/ Chick (g.)	Mean Weight Gain/ Chick (g.)	Avg. Feed Consumed/ Chick (g.)	
Control	40.3	26.9	50.4	79.7	95.3	1
Control[b]	39.5	25.9	63.8	78.8	100.5	3
9.5	39.6	24.4	57.0	78.8	89.6	1
19.2	39.6	25.3	57.1	73.6	89.2	0
38.3	39.4	25.3	58.1	77.1	87.2	1
76.6	39.1	22.6	59.0	71.1	85.8	0
153.0	40.4	24.1	55.9	71.2	86.6	1
306.0	38.7	21.2	53.0	72.3	87.1	3
613.0	38.6	14.3	36.2	48.9	80.1	1

[a] 40 chicks/treatment at start of test.
[b] Wettable powder formulation ingredients without Guthion.

p.p.m. level, total intake decreased proportionally with insecticide concentration and that feed intake was very low at the 2,177 p.p.m. level. It appeared that: (1) Starvation played a part in the high mortality from 2,177 p.p.m., and (2) this concentration of Guthion approached the acute toxicity level.

Experiment 2 confirmed the previous finding that concentrations of Guthion up to 153 p.p.m. did not cause depression in weight gains. Table 2 shows that the first apparent depression in gains in weight occurred at 306 p.p.m. There was no apparent difference between the regular and the "carrier" controls, indicating that any adverse effects were caused by Guthion rather than the other ingredients in the insecticide formulation. None of the treatments showed a mortality in excess of that of the control in this experiment.

Analysis of variance of the data obtained in Experiment 3 showed a highly significant ($P < 0.01$) depression in gain in weight in those groups receiving 600 p.p.m. Guthion (table 3). No significant interactions were evident when Guthion and either of the two coccidiostats were combined in the basal ration. It was also noted that neither the coccidiostats nor the 100-p.p.m. level of Guthion, either alone or in combination, significantly affected the mean weight gains.

The analyses of stored feed for the presence of Guthion showed that although the rate of loss of Guthion was inversely proportional to the initial concentration, the actual amounts of insecticide lost increased proportionally with initial concentrations (table 4). In Experiments 1 and 3, the rations were prepared in 4-pound lots at least twice weekly and consumed quite rapidly. However, in Experiment 2 the initial 20-pound mixtures were sufficient for the 2-week test period. Therefore in this experiment there was time for considerable loss of insecticide during storage, particularly at the higher concentrations. There is an indication of such loss in the weights for the chicks fed at the 306 p.p.m. level shown in table 2. The gains of these chicks were obviously depressed during the first week but apparently normal during the second week. Although the chicks may have attained some degree of adaptation to the insecticide with increased age, loss of Guthion from the feed appears to be a more logical explanation for these results.

Table 3.—Effects of two levels of Guthion alone and in combination with two different coccidiostats in starter feed when fed to day-old, male broiler chicks over a 2-week period.

Treatment		Initial[a] Mean Weight (gm.)	Mean Weight Gain (gm.)		No. Dead at End of Test
Guthion (p.p.m.)	+ Coccidiostat		1 week	2 week	
0	0	38.1	106.5	168.3	0
0	N[b]	36.2	96.9	159.8	1
0	SQ[c]	35.8	100.6	166.5	0
100	0	36.5	97.7	163.4	0
100	N	37.8	98.7	155.7	0
100	SQ	36.6	95.0	155.1	0
600	0	36.4	75.0	102.2	0
600	N	35.7	73.0	100.4	1
600	SQ	37.3	78.1	107.2	1

[a] 30 chicks/treatment at start of test.
[b] Nicarbazin, 0.0125%.
[c] Sulfaquinoxiline, 0.0175%.

Table 4.—Disappearance of Guthion in chick starter feed after storage for 2 weeks in metal containers.

Initial Concentration[a] (p.p.m.)	After 2 Weeks[b] (p.p.m.)	Loss in Weight (p.p.m.)	Loss (%)
0	0	—	—
77	40	37	48.1
153	88	65	42.1
307	190	117	38.1
613	400	213	35.5

[a] Calculated level.
[b] Amount determined by chemical assay.

CONCLUSIONS.—The following conclusions are presented on the basis of the work reported here: (1) Baby chicks are not sufficiently sensitive to low levels of Guthion in starter feed to warrant their use as a bioassay organism for this insecticide. (2) Owing to the fairly rapid rate of disappearance of Guthion from feed, and the relatively large amounts of it required to affect gains in weight of young chicks, it seems highly unlikely that field use of the insecticide at the recommended dosages would ever lead to its appearance in commercial feeds in amounts sufficient to produce obvious toxic symptoms in baby chicks. (3) At the levels tested, no interaction was detected between Guthion and two commercial coccidiostats, Nicarbazin and Sulfaquinoxiline, used at recommended dosages.

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Metabolism of *O,O*-Dimethyl *O*-[4-(Methylthio)-*m*-Tolyl] Phosphorothioate by White Rats[1]

U. E. Brady, Jr., and B. W. Arthur,
Department of Zoology-Entomology, Auburn University, Auburn, Alabama

Abstract

O,O-dimethyl *O*-[4-(methylthio)-*m*-tolyl] phosphorothioate or Bayer 29493 was oxidized by rats at the phosphoryl sulfur and the thiophenyl group; the sulfoxide and sulfone derivatives of the parent material and its oxygen analog were isolated and identified. Oxidation rather than isomerization was the predominant activation process. The urine and feces contained different percentages of each metabolite. Hydrolysis occurred primarily at the P-O-phenyl bond; cleavage of the P-O-methyl bond was not demonstrated. The percentage of hydrolytic products in the urine decreased as the number of doses increased (10 mg./kg./day for 10 days). The cholinesterase of the blood and brain of rats was inhibited rapidly and recovered slowly.

The acetonitrile-soluble residues in the liver, kidney, muscle, skin, and heart were negligible at 3 days following oral (100 mg./kg.) or intraperitoneal treatment of rats. About 80% of the administered Bayer 29493 equivalents was eliminated in the excreta regardless of the route of administration.

O,O-dimethyl *O*-[4-(methylthio)-*m*-tolyl] phosphorothioate, referred to in this paper by its code number Bayer 29493, is effective against household and medically important insects, such as the house fly (*Musca domestica* L.), the triatomid (*Rhodnius prolixus* Stahl.), and the mosquito (*Culex p. quinquefasciatus* Say) (Blazquez 1959). The residual effectiveness of Bayer 29493 against these insects was several days, depending upon the nature of the treated surface. This phosphorothioate was effective against the bed bug (*Cimex lectularius* L.), but was less effective against the Gulf Coast tick (*Amblyomma maculatum* Koch) in animal systemic screening tests, using rabbits treated orally at 25 mg./kg. (Brady et al. 1960). The practical use of Bayer 29493 for livestock pest control appears promising (MacDougall 1960). Bayer 29493 has a favorable rat-to-house fly toxicity ratio (Brady et al. 1960).

Results of metabolism studies of Bayer 29493 on white rats following single oral administrations or multiple intraperitoneal injections are reported in this paper. Preliminary studies were also made on the metabolism of Bayer 29493 by certain insects and the cotton plant.

Procedure.—*Materials.*—Radiophosphorus Bayer 29493 (I) and the following oxidation or isomerization products of the parent compound were used in this study: (II) *O,O*-dimethyl *O*-[4-(methylsulfinyl)-*m*-tolyl] phosphorothioate, the sulfoxide of Bayer 29493; (III) *O,O*-dimethyl *O*-[4-(methylthio)-*m*-tolyl] phosphate, the oxygen analog of Bayer 29493; (IV) *O,O*-dimethyl *O*-[4-(methylsulfinyl)-*m*-tolyl] phosphorothioate, the sulfone of Bayer 29493; (V) *O,O*-dimethyl *O*-[4-(methylsulfinyl)-*m*-tolyl] phosphate, the sulfoxide of the oxygen analog; (VI) *O,O*-dimethyl *O*-[4-(methylsulfinyl)-*m*-tolyl] phosphate, the sulfone of the oxygen analog; and (VII) *O*-methyl *S*-methyl *O*-[4-(methylthio)-*m*-tolyl] phosphate, the *S*-methyl isomer of Bayer 29493 These six suspected metabolites of Bayer 29493 were chromatographed on Celite and over 95% of the phosphorus-containing materials were eluted as a single fraction. Radioactive Bayer 29493 was chromatographed on a Celite partition column and the principal eluate fraction yielded an infrared spectrogram identical to a known column purified sample of nonradioactive Bayer 29493. The solvent system used to develop the chromatograms was the same as reported by Boyd & Arthur (1960) except that the amount of chloroform added to the mixture of *n*-hexane and acetonitrile mobile phase varied from 1% to 3%.

Excretion and Metabolism by Rats.—Three groups of rats were treated with P^{32}-labeled Bayer 29493. The first group of rats, designated as Group A, consisted of 18 males weighing from 150 to 175 g. each. Rats were treated intraperitoneally at 10 mg. of Bayer 29493 per kg. of body weight per day for 10 consecutive days. The appropriate mg./kg. dosage of Bayer 29493 was dissolved in 0.5 ml. of a 20:80 mixture of ethanol and propylene glycol and was injected with a 1-cc. syringe equipped with a No. 25 gauge needle. The rats were confined in round metabolism cages (3 rats per cage), which permitted separate collections of urine and feces. Groups of three rats each were sacrificed at 1, 3, 7, 10, 13, and 20 days after beginning the experiment.

The liver, kidney, heart, and a portion of the skin, muscle, and bone were removed from the rats at each sacrificing time interval and the total P^{32} materials was determined as described by Boyd & Arthur (1960). From 1 to 5 g. of each tissue, except the bone, was extracted with acetone and benzene. The aqueous residue of these extracts was partitioned with *n*-hexane, then the *n*-hexane was extracted with an equal volume of acetonitrile (Anderson et al. 1959). Aliquots of each tissue fraction were assayed for total radioactivity. Recoveries of Bayer 29493 (0.1 to 1.0 p.p.m.) from control liver and muscle using the extraction procedure described above were 85% and 91% respectively. The cholinesterase activity of the blood and brain was measured colorimetrically according to the method of Robbins et al. (1958).

Urine and fecal samples from each cage of rats were collected at 24-hour intervals throughout the 20-day experimental period. The p.p.m. Bayer 29493 equivalents and the cumulative percentage of the administered dose eliminated in the excreta were calculated (Boyd & Arthur 1960). The urine was partitioned three times with an equal volume of chloroform to separate the Bayer 29493 hydrolysis products from the parent material or its oxidative metabolites. Ten grams of feces collected at 24-hour

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intervals from each replicate of rats was ground to a powder with a mortar and pestle and placed in a flask containing 100 ml. of water and allowed to stand for 24 hours. The water was decanted and 100 ml. of acetone was added to the feces. The water and acetone extracts were combined and extracted three times with chloroform. Two 0.1-ml. aliquots of the water and the chloroform were assayed to determine the total P^{32} materials in each layer. All of the P^{32} materials that remained in the water after chloroform extraction were considered as hydrolytic products and were chromatographed on an anion exchange column with nonradioactive *O,O*-dimethyl phosphoric and *O,O*-dimethyl phosphorothioic acids (Plapp & Casida 1958b). Recoveries of Bayer 29493 (1.0 p.p.m.) added to control urine and feces were 85%.

A second group of rats (Group B) consisted of two male rats treated intraperitoneally with a single dose of Bayer 29493 at 200 mg./kg. At the onset of symptoms of poisoning, about 1.5 hours after treatment, the rats were sacrificed and the cholinesterase activity of the blood and brain and the p.p.m. Bayer 29493 equivalents in several tissues were determined as described for the Group A rats.

The third group of rats (Group C) consisted of two replicates of two male rats each that were treated orally with Bayer 29493 at 100 mg./kg. The toxicant was dissolved in 0.5 ml. of corn oil and administered through a polyethylene stomach tube. Two rats were sacrificed on the third day and two on the seventh day following treatment. The total p.p.m. Bayer 29493 equivalents in several tissues and the chloroform-soluble P^{32} materials were determined by methods described previously (Boyd & Arthur 1960). The urine and feces were collected daily and processed in a similar manner as the excreta from Group A rats. The urine and the water-solubles from the feces were extracted six times with an equal volume of chloroform. The hydrolytic products from Bayer 29493 degradation were chromatographed on an anion exchange column. The chloroform solubles were chromatographed on a Celite partition column with the suspected oxidative metabolites of Bayer 29493.

In vitro Metabolism.—The effect of beta-diethylaminoethyldiphenylpropylacetate hydrochloride (coded as SKF 525A), an antidote for certain organophosphates (Davidson 1955), on the activation and detoxification of Bayer 29493 and Bayer 22408 (*O,O*-diethyl-*O*-naphthalimido phosphorothioate) by rat liver was studied *in vitro* using the methods of DuBois et al. (1950). One gram of thinly sliced rat liver was added to each of three flasks containing 10 ml. of physiological solution, 3 mg. of the organophosphate, and 1.5 mg. of SKF 525A. Another three flasks contained only the organophosphate and rat liver in physiological solution. The flasks were incubated in an oxygen atmosphere on a metabolic reciprocating shaker for 4 hours. The physiological solution was decanted and extracted three times with chloroform. The percentage hydrolysis was calculated from the amount of radioactivity in the water and that remaining in the liver residue. The chloroform-soluble materials were subjected to Celite chromatography for identification of oxidative metabolites.

Insect and Plant Metabolism Studies.—The metabolism of Bayer 29493 was studied in the house fly, German cockroach (*Blattella germanica* (L.)), and boll weevil (*Anthonomus grandis* Boheman). Two groups of 150 adults of each insect species were treated topically with 25 μg. of Bayer 29493 per g. of insect. At 1 and 4 hours after treatment, the insects were surface-washed with acetone to remove unabsorbed or excreted insecticide. They were macerated in 50 ml. of water followed by 50 ml. of acetone in a Waring Blendor equipped with a microhead. The insect brei was filtered through four thicknesses of cheesecloth and the water portion (after removal of the acetone by evaporation on a steambath) was extracted six times with an equal volume of chloroform. The chloroform extracts were combined and processed for Celite partition chromatography as described for the chloroform-solubles from rat excrement.

Cotton plants were sprayed with a xylene-Triton X-155-water emulsion of Bayer 29493 at the rate of 2 pounds active ingredient per acre. The plants were 24 to 30 inches high, fruiting freely, and growing under field conditions. Leaves from the plants were assayed for total Bayer 29493 equivalents and then fractionated into water-soluble and chloroform-soluble radioactive materials according to methods described by Boyd & Arthur (1960). Water-solubles were chromatographed on an anion exchange resin and chloroform-solubles on a Celite partition column with known nonradioactive degradation products of Bayer 29493 for characterization of metabolites.

Table 1.—P.p.m. Bayer 29493 equivalents in tissues of rats treated intraperitoneally or orally.

Dosage and Time After Treatment	P.p.m. Bayer 29493 Equivalents in—[a]					
	Liver	Kidney	Bone	Muscle	Skin	Heart
10 mg./kg./day for 10 days, IP[b]						
3 days	12.7	7.2	14.8	1.4	4.5	3.0
7 days	14.5	12.9	13.3	5.5	6.5	5.9
10 days	12.4	9.4	60.2	4.2	6.3	6.6
13 days	12.6	10.3	49.7	6.7	4.9	8.6
20 days	4.1	2.9	51.4	3.4	2.1	3.2
200 mg./kg. Single IP[c]						
1.5 hours	124.4	91.7	22.3	52.6	25.3	20.8
100 mg./kg. Single oral[c]						
3 days	34.1	24.6	—	12.1	12.1	13.1
7 days	12.4	8.3	—	6.9	3.8	6.3

[a] See text for p.p.m. acetonitrile-soluble residues.
[b] Averaged from 3 rats at each time interval.
[c] Averaged from 2 rats at each time interval.

RESULTS.—*Mammalian Studies.*—Bayer 29493 equivalents in several tissues of rats following intraperitoneal or oral treatment are given in table 1. The accumulation of P^{32} materials in the liver and kidney followed the same distribution pattern observed with other phosphorothioates, such as ronnel (Plapp & Casida 1958a), Co-Ral (Krueger et al. 1959, Vickery & Arthur 1960) and Bayer 22408 (Boyd & Arthur 1960). The bone contained a large amount of Bayer 29493 equivalents even after a relatively brief period following treatment, and the amount increased quite rapidly when Bayer 29493 was administered at 10 mg./kg./day for 10 days (table 1). The incorporation of P^{32} in bone was further evidence that Bayer 29493 was rapidly degraded by rats. The acetonitrile-soluble radioactive residues in the tissues following multiple injections of Bayer 29493 were below the limit of sensitivity of the radioassay method (0.05 p.p.m.). Bayer 29493 or its oxidative metabolites were not stored in tissues even when rats received daily doses of 10 mg./kg. for 10 consecutive days. Acetonitrile-soluble residues were detectable in tissues of rats at 1.5 hours following a single intra-

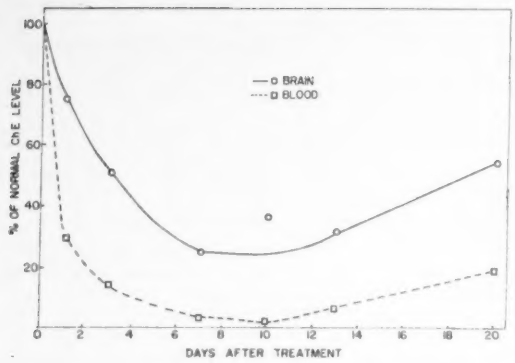

FIG. 1.—Effect of Bayer 29493 on the cholinesterase level of the blood and brain from rats treated intraperitoneally at 10 mg./kg./day for 10 days.

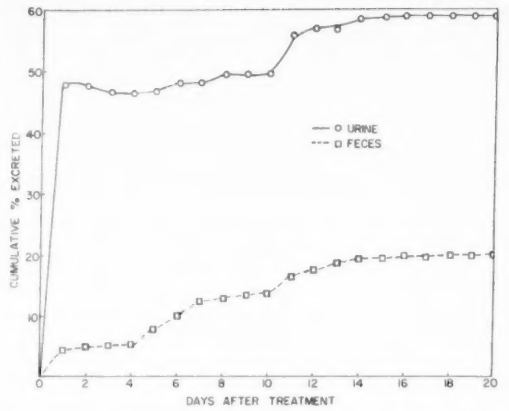

FIG. 2.—Cumulative percentage of the administered Bayer 29493 equivalents excreted in the urine and feces of rats treated intraperitoneally at 10 mg./kg./day for 10 days.

peritoneal injection of 200 mg./kg.; the following p.p.m. acetonitrile-soluble residues were observed: liver, 29.5, muscle 9.6, skin 6.1, kidney 26.9, and heart 9.6. The liver from rats treated orally at 100 mg./kg. contained 0.2 p.p.m. chloroform-soluble residues at 3 days after treatment and all other tissues contained less than 0.01 p.p.m. The blood, brain, and fat from rats treated orally contained no detectable acetonitrile-soluble residues at 3 or 7 days' posttreatment.

Cholinesterase in whole blood was inhibited to a greater extent than the cholinesterase in the brain of rats. The lowest level in the blood was on the last day of treatment at 10 mg./kg./day intraperitoneally (fig. 1). The return to normal cholinesterase levels was slow. The cholinesterase levels of the blood and brain of rats receiving a single intraperitoneal injection of 200 mg./kg. was 2.0% and 31.0%, respectively, at 1.5 hours after treatment.

The cumulative percentage of the administered doses that was eliminated in the excreta of rats treated intraperitoneally or orally is shown in figures 2 and 3. Rats treated orally eliminated 86.2% of the radioactivity in the excreta by 7 days after treatment (fig. 3), whereas rats treated intraperitoneally eliminated 79.5% by 20 days after the first injection of 10 mg./kg. (fig. 2). Although these combined cumulative percentages (urine plus feces) were about the same, the feces from rats treated orally contained considerably more P^{32} materials than the feces from rats treated intraperitoneally. Apparently, Bayer 29493 was not absorbed very readily through the intestinal tract.

The hydrolysis of Bayer 29493 by rats treated with multiple injections followed a peculiar pattern in that, as the number of doses administered increased, the proportionate amount of hydrolytic products in the urine decreased (fig. 4). This situation continued for 3 days past the last injection. From 96% to 99% of the radioactive materials in the urine or feces of orally treated rats were hydrolysis products by 3 days after treatment at 100 mg./kg. Results of chromatographic studies of hydrolytic products of the urine and feces are given in table 2. There was little difference in the percentage of each metabolite in the urine collected at the various intervals after treat-

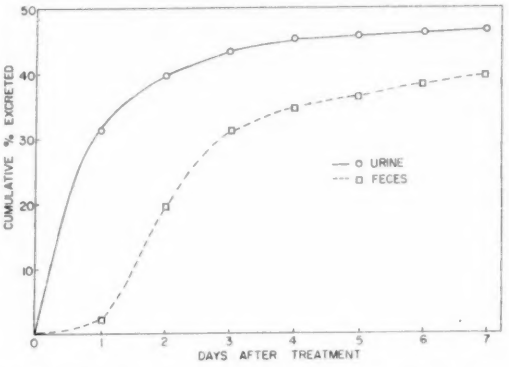

FIG. 3.—Cumulative percentage of the administered Bayer 29493 equivalents excreted in the urine and feces of rats treated orally at 100 mg./kg.

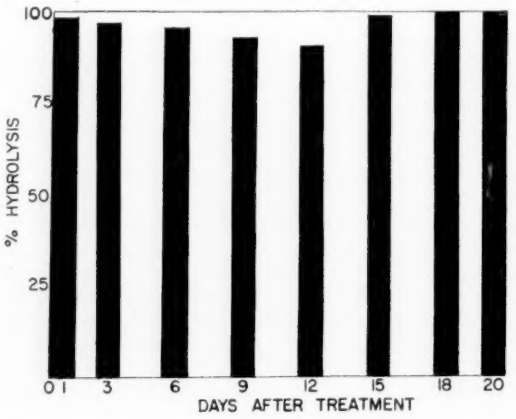

FIG. 4.—Percentage hydrolysis products of Bayer 29493 in the urine of rats treated intraperitoneally at 10 mg./kg./day for 10 days.

ment. The predominant hydrolytic product of the urine was *O,O*-dimethyl phosphorothioic acid while the *O,O*-dimethyl phosphoric acid was the major constituent of the feces. Two unidentified hydrolytic metabolites were recovered from the urine of intraperitoneally treated rats. The des-methyl derivative of the parent compound or one of its oxidation products is a possible explanation of these metabolites.

The total radioactivity in the urine and feces from rats treated orally that was extractable from water into chloroform (1% to 4%) consisted of several oxidation products of Bayer 29493 (table 3). The phosphoryl sulfur and the thiophenyl moieties underwent oxidation to form the sulfoxide (V) and the sulfone (VI) of the oxygen analog of Bayer 29493. These two metabolites were not well separated on Celite, but constituted the major oxidative metabolites of the urine. The oxygen analog (III) of Bayer 29493 was the predominant chloroform-soluble metabolite of the feces. The feces contained more unchanged Bayer 29493 than the urine. The sulfoxide (II) and the sulfone (IV) of Bayer 29493 were not available when the studies were made with rats treated orally, but tentative identifications were made based on their chromatographic behavior. The other metabolites given in table 3 were available for co-chromatographic identifications. The methanol fraction from Celite represents the percentage of radioactivity that could not be eluted with *n*-hexane saturated with acetonitrile plus 3% chloroform, and were not studied further. The chloroform-soluble materials recovered from rats receiving intraperitoneal injections were not sufficient for characterization by Celite partition chromatography.

In vitro Liver Metabolism.—SKF 525A appeared to facilitate the hydrolysis of Bayer 29493 and Bayer 22408 by rat liver slices. About 33% of the Bayer 29493 was hydrolyzed after incubation with rat liver for 4 hours whereas 38% was hydrolyzed by liver slices in the presence of SKF 525A. The percentage hydrolysis of Bayer 22408 was 22% without SKF 525A and 26% with SKF 525A. When these two phosphorothioates were incubated without rat liver, less than 2% of the radioactivity appeared as hydrolytic products regardless of whether SKF 525A was added.

The chloroform-soluble radioactive materials recovered from liver slices chromatographed mostly as the parent compound (56% to 61%). In the presence of SKF 525A, the sulfone (IV) of Bayer 29493 constituted 38% of the total radioactive materials as compared with 23% when Bayer 29493 was incubated with liver slices in the absence of SKF 525A. SKF 525A had no effect in preventing the *in vitro* oxidation of Bayer 22408 or Bayer 29493.

Insect and Plant Metabolism.—The absorption and stability of Bayer 29493 in three species of insects are given in table 4. Bayer 29493 was absorbed more readily by the house fly at 4 hours after treatment than by the German cockroach or boll weevil. A greater percentage of the absorbed dose was hydrolyzed by the boll weevil than by the other two insect species.

The nonhydrolyzed radioactive materials were largely unchanged Bayer 29493 (table 3). However, insects were capable of oxidizing the thiophosphate and thiophenyl groups to form the five possible oxidation products of the parent material. The proportion of each oxidative metabolite was quite variable with the three insect species.

Table 2.—Hydrolysis products of Bayer 29493 in rat excrement.

Dosage and Days After Treatment	Per Cent of Water-Soluble Radioactivity—			
	$(CH_3O)_2P(O)OH$	Peak 2[a]	$(CH_3O)_2P(S)OH$	Peak 4[a]
10 mg./kg./day for 10 Days, IP				
1 (urine)	41.3	2.8	52.0	3.9
3 (urine)	43.9	5.2	47.2	3.9
7 (urine)	28.1	2.9	65.8	2.3
10 (urine)	38.0	6.7	53.0	2.3
100 mg./kg. Single oral				
1 (urine)	19.5	—	80.5	—
2 (urine)	27.6	—	72.4	—
3 (urine)	35.0	—	65.0	—
1–2 (Feces)	83.0	—	17.0	—

[a] Peaks 2 and 4 refer to the order of elution from the column and were not identified. Dimethyl phosphoric acid was peak 1 and the thioic acid was peak 3.

Table 3.—Fractionation by Celite partition chromatography of the chloroform-soluble radioactive materials recovered from rat excrement, insects, and cotton plants.

Biological System	Time After Treatment	Per Cent of Chloroform-Soluble Radioactivity—[a]					
		Peak I	II	III	IV	V & VI	MEOH
Rat (urine)[b]	1 day	6.5	3.7	1.3	0	87.4	1.1
	2 days	2.2	3.6	2.7	0	86.5	5.0
	3 days	2.2	7.7	21.4	6.4	57.4	4.9
Rat (feces)	1–3 days	22.4	0	53.5	0	14.3	9.8
House fly	1 hour	63.5	5.5	7.8	18.3	4.9	0
	4 hours	44.3	5.7	3.3	18.6	26.3	1.8
German cockroach	1 hour	91.1	0	0	6.0	3.0	0
	4 hours	52.7	.5	3.1	31.5	11.9	.3
Boll weevil	4 hours	48.6	20.5	18.4	8.5	4.0	0
Cotton plant	3 days	27.5	.8	1.2	62.2	7.6	.7
	7 days	12.7	.6	.2	76.4	9.5	.6
	14 days	13.1	0	.7	78.5	5.6	2.1

[a] (I) Bayer 29493, (II) the sulfoxide of Bayer 29493, (III) the oxygen analog of Bayer 29493, (IV) the sulfone of Bayer 29493, (V) the sulfoxide of the oxygen analog, and (VI) the sulfone of the oxygen analog. See text for interpretations of tentative identifications. Peak numbers refer to the order of elution from the column.

[b] Rats treated orally at 100 mg./kg.

Table 4.—Absorption and stability of Bayer 29493 in insects.

Insect	Hours After Treatment	Per Cent of Applied Dose Absorbed	Per Cent of Absorbed Dose Hydrolyzed
House Fly	1	27.4	34.8
	4	64.4	56.2
German cockroach	1	44.1	13.7
	4	49.1	39.3
Boll weevil	4	42.6	76.5

Bayer 29493 was degraded rapidly by cotton plants. By 3 days after spraying, 189 p.p.m. Bayer 29493 equivalents were present on or in the leaves, but only 14 p.p.m. were chloroform-soluble materials. The residues at 14 days posttreatment were 71 p.p.m. total P^{32} materials and 5 p.p.m. chloroform-soluble materials. The chloroform-soluble materials contained the parent material and its five possible oxidation products (table 3). The sulfone (IV) of Bayer 29493 was by far the most prevalent metab-

olite in plants. This identification was tentative and was based on the order of elution from the column and was not based on co-chromatographic properties, since the known material (IV) was not available when the plant studies were made. The hydrolytic products were separated into dimethyl phosphorothioic acid (85%), dimethyl phosphoric acid (10%), and an unknown metabolite (5%). The ratio of these acids remained relatively constant throughout the 14-day experimental period.

DISCUSSION.—Bayer 29493 underwent thiophosphate and thiophenyl oxidation forming five oxidative metabolites in rats, insects, and cotton plants (table 3). However, the proportion of the oxidative metabolites was vastly different for the three biological systems, and there were differences between insect species as well as the excreta of rats. The metabolic pathway of this phosphorothioate was the same as other phosphorothioates and phosphorodithioates having a thioether group (Fukuto et al. 1955, Bowman & Casida 1957, Metcalf et al. 1957). There was no evidence that the *S*-methyl isomerization product (*O*-methyl *S*-methyl *O*-(4-(methylthio)-*m*-tolyl) phosphate) was formed *in vivo* as was demonstrated for Bayer 25141 (*O,O*-diethyl *O*-*p*-methyl-sulfinylphenyl phosphorothioate) (Benjamini et al. 1959). The *S*-methyl isomer is a possible metabolite and its absence was based on the order of elution from Celite and not by its co-chromatographic behavior with radioactive unknowns (table 3). The sulfone (IV) and sulfoxide (II) derivatives of Bayer 29493 were not available when most of the metabolism studies were conducted (table 3); the identity of these metabolites was tentative.

Bayer 29493 was hydrolyzed at the P—O—C bond to form dimethyl phosphorothioic acid and the presence of dimethyl phosphoric acid was probably due to hydrolysis of the oxygen analog of Bayer 29493. The unidentified water-soluble metabolites isolated by anion exchange chromatography could have been the des-methyl derivative of the parent compound or the oxygen analog. Cleavage of the *O*-methyl ester of this phosphorothioate appeared to be a minor degradative mechanism.

The hydrolysis of Bayer 29493 by rats receiving multiple intraperitoneal injections followed a peculiar pattern as compared with other organophosphates (Arthur & Casida 1959). As the number of Bayer 29493 doses increased, the percentage hydrolysis products in the urine of rats decreased (fig. 4). Perhaps there was an inactivation of the enzymes necessary for complete hydrolysis of this organophosphate. Although Bayer 29493 was not stored to any appreciable extent in tissues, the incomplete hydrolysis probably allowed a sufficient quantity of anti-esterase agent(s) to reach the final site of action and cause death or symptoms of poisoning. The decrease in the hydrolysis rate as the number of doses of Bayer 29493 increased may be an explanation of the apparently prolonged chronic toxicity of the compound (MacDougall 1960).

SKF 525A, facilitated the hydrolysis of Bayer 29493 and Bayer 22408 to a slight extent but was not effective in preventing *in vitro* oxidation of these two phosphorothioates. SKF 525A has been reported to inhibit oxidative processes of parathion and schradan (Davidson 1955). Increased hydrolysis of Bayer 29493 by rat liver in the presence of SKF 525A was similar to the action of certain pyridine aldoxine methiodides on other organophosphates (Gatterdam et al. 1959).

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Field Studies on the Toxicity of Insecticides to the Mosquito Fish, *Gambusia affinis*[1]

MIR S. MULLA,[2] *Department of Entomology, University of California, Riverside, and* LEWIS W. ISAAK,[2] *Kern Mosquito Abatement District, Bakersfield, California*

ABSTRACT

The toxicity of 16 insecticides against the mosquito fish, *Gambusia affinis* (Baird and Girard), was studied in the field. All the toxicants were applied at two to four dosages. The lower dosages approximated the probable maximum rates of toxicants used currently or which might be used in the future as mosquito larvicides. The higher dosages were four times the lower dosages, and these higher rates were considered to approximate the probable maximum dosages to be administered for the control of pests of agricultural crops.

Bayer 29493 (Baytex®) (*O,O*-dimethyl *O*-[4-methylthio)-*m* tolyl] phosphorothioate), Bayer 25198 (*O,O*-dimethyl *O*-*p*-methylsulfinylphenyl) phosphorothioate), Dowco 139 or Zectran® (4-dimethylamino-3,5-xylyl methylcarbamate), AC-5727 (*m*-isopropylphenyl *N*-methylcarbamate), and Dow *K*-6882 (*O*-ethyl *O*-(2,4,5-trichlorophenyl) methyl phosphoromidothioate) at lower and higher dosages resulted in slight or no mortality of fish confined for 48 hours or longer in the treated plots. These materials are considered safe against the fish at the indicated dosages.

Malathion, DDT and Methyl Trithion® [*O,O*-dimethyl *S*(*p*-clorophenyl) thiomethyl phosphorodithioate] at high and low rates resulted in appreciable mortality of the fish within a period of 48 hours of exposure. Parathion at 0.1 pound per acre caused 30% mortality of the fish 48 hours after exposure. Beyond this period no mortality was produced by this dosage. At 0.4 pound per acre parathion produced a high kill of the fish initially and appreciable mortality was obtained at this dosage up to 6 days after treatment. At the lower dosages DDT and parathion are somewhat safer than malathion and Methyl Trithion.

Bayer 25141 (*O,O*-diethyl *O*-*p*-(methylsulfinylphenyl)phosphorothioate) (except at the lower dosage), Bayer 38156 (*O*-ethyl *S*-*p*-methylphenyl ethylphosphonodithioate), American Cyanamid E.N. 18133 (*O,O*-diethyl *O*-2-pyrazinyl phosphorothioate), GC-3583 (diethyl-1-(2,5-dichlorophenyl)-2-chlorovinyl phosphate), GC-4072 (diethyl-1-(2,4-dichlorophenyl)-2-chlorovinyl phosphate), and SD-4402 (1,3,4,5,6,7,8,8-octachloro 3a,4,-7,7a-tetrahydro-4,7-methanophthalan), proved highly toxic to the fish initially at all dosages tested. The initial toxicity of Bayer 25141 at the lower dosage (0.2 pound/acre) was moderate and comparable to that of malathion; the toxicity of both declined sharply after 48 hours. All these materials at the higher dosages had long residual toxicity against the fish, but GC-3583, E.N. 18133 and Bayer 25141 had the most residual toxicity at the higher dosages. Dekafos® (3-pentadecylphenol *O-O*-diethylthiono phosphate) manifested only moderate initial toxicity.

The mosquito fish, *Gambusia affinis* (Baird and Girard), lives in a complex ecosystem in association with a large number of plant and animal species. It is a voracious feeder and consumes a variety of phytoplankton, zooplankton, as well as some relatively larger aquatic insect species. It shows cannibalistic tendencies and provides food for larger vertebrates such as fishes, birds and others. Notwithstanding these factors, the fish build heavy populations in permanent and semipermanent bodies of water.

Gambusia affinis was described about a century ago but its usefulness as an enemy of mosquito larvae was pointed out only about 50 years ago (Seal 1908). Three years later, Vasilyev and Porchinsky, as reported by Sokolov (1958), pointed out the possibility of using *Gambusia* fish in antianopheline programs in Russia. Hildebrand (1925) demonstrated marked reduction of anopheline and culicine larvae and pupae in bodies of water containing *Gambusia affinis holbrooki* compared with water devoid of the fish. This is a detailed study of the relationship of *Gambusia* to mosquito larval populations. Weed (1924) suggested that microcrustaceans, through effective competition for food, could reduce mosquito larval populations to a level where the mosquito fish could eliminate the surviving individuals. Subsequently, Hintz (1951) observed copepods feeding on larvae of *Aedes aegypti* in a laboratory aquarium, and suggested that this might occur under natural conditions just as well. However, Hess & Tarzwell (1942) showed that *Gambusia* feed on Entomostraca through necessity, and prefer macroscopic organisms.

In anti-malarial activities, the mosquito fish has been utilized as an adjunct to larvicidal and source reduction programs in the Old World (Ciuca 1956, Petragnani & Castelli 1927, Sokolov 1958) and the New World (Neghme et al. 1955). Hixson (1943) observed *G. affinis* to be the principal predator of *Anopheles* larvae in certain associations in Florida. Rees (1958) obtained complete control of mosquito larvae in ponds where the fish were added at the rate of 1 to 3 per square yard. This work includes a study of the feeding activity and ecological behavior of the fish. More than 50 species of plants and animals were consumed by the fish. Hess & Tarzwell (1942) demonstrated that *Gambusia* forage ratios for a number of arthropods (including anopheline and culicine larvae) increased as the population density of the organisms increased in the aquatic habitat.

For stocking streams, *Gambusia affinis* was introduced into Hawaii in the early part of this century (Seal 1908). It has also been introduced and established in Utah and Alberta, where subfreezing temperatures prevail for a few months of the year (Rees 1934, Mail 1954). A *Gambusia* fish was introduced into the U.S.S.R. in 1928 from Italy, and acclimatization studies in the U.S.S.R. have pointed out the hardiness of the fish (Sokolov 1958). It is now

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widely spread in central Asia, the Transcaucasia, and has spread into Iran. Attempts are now being made to establish this viviparous predator in the northern portions of the U.S.S.R.

The mosquito larval predators of the genus *Gambusia* have a worldwide distribution and probably play an important role in suppressing the active immature stages of mosquitoes either by feeding directly on the active, immature stages of the pests or by creating unfavorable physical environment owing to their constant movement. Seal (1908) observed that female mosquitoes (with the exception of *Anopheles*) do not lay eggs in bodies of water that have fish or other predators. The present authors noticed that *Gambusia affinis* (the widespread species in the United States) does not readily feed on the very young larvae, and greater reductions of populations have been observed in the later stages of the development of the larvae and pupae. This supports the work of Hess & Tarzwell (1942).

Gambusia affinis is abundant in California and breeds successfully in many types of permanent and semipermanent bodies of water where various species of mosquitoes breed in large numbers. Many of the California Mosquito Abatement Districts routinely stock bodies of water such as ponds, streams, reservoirs, animal troughs, et cetera, where the fish are likely to survive. Once introduced into an optimum environment, the fish multiply rapidly and suppress mosquito breeding or larval and pupal populations, particularly when they are associated with other predaceous aquatic insects. It has been considered one of the major factors aiding in the suppression of mosquito larval and pupal populations in rice fields. The equilibrium level of fish and other predators, however, may be disrupted at times by the use of mosquito larvicides or agricultural pest control operations over or adjacent to water bodies where the fish are thriving.

Although the mosquito fish is considered a major predator of mosquito larvae, treatment of mosquito breeding sources becomes necessary at times even though a fair number of the fish are present. A selective material which will result in complete kill of the larvae at economic dosages and spare the fish population would be of utmost advantage. Short-lived materials, even though with appreciable initial toxicity to the fish, might be used in situations where immediate introduction of the fish into the breeding source is contemplated after treatment for larval control.

Numerous laboratory and field studies and observations on the effect of toxic chemicals, including insecticides and solvents used for formulating insecticides, on fish other than *Gambusia* have been reported (Cope et al. 1947, Davidow & Schwartzman 1955, Linduska & Surber 1948, Springer & Webster 1951, Surber 1951, Tarzwell 1950). There is a general lack of information on the initial and residual toxicity of mosquito larvicides and other insecticides to the mosquito fish. Lewallen (1959) studied the initial toxicity of several organophosphorus insecticides in the laboratory. Among these, malathion and parathion, the most commonly used larvicides in California at the present time, proved to be moderately toxic to the fish where the fish were exposed to the toxicants for 24 hours.

In the present study, an attempt was made to determine the toxicity of various insecticides to the mosquito fish in the field. Emphasis was placed on those insecticides which are currently employed as mosquito larvicides in California. Materials with great potential for use in agricultural pest control and mosquito abatement programs were also considered in these studies. The residual toxicity of stable materials to the fish was extensively studied.

EXPERIMENTAL PROCEDURE.—The studies were undertaken in water ponds (fig. 1) constructed and maintained by the Kern Mosquito Abatement District. The ponds are located in a predominantly agricultural area near Bakersfield, California, where intensive mosquito control activities have been carried on for the past several years. The ponds are arranged in a row, each pond being 1/16 acre in size. The water pH in the ponds was 7.5 to 8.0 and the temperature during the tests fluctuated from 63° to 94° F. The materials listed in table 1 to 3 were evaluated during August and September, 1960, when the water temperature fluctuated from 78° to 94° F. The materials listed in table 4 were studied during October, and studies on the residual activity of some of these materials were continued into November, when the water temperature de-

Table 1.—Toxicity of insecticides to *Gambusia affinis* in the field.[a]

Material and Formulation	Toxicant (lb./acre)	Per Cent Mortality of Fish at Indicated Intervals After Treatment								
		Hours				Days				
		24	48[b]	72	96	5	6	7	8	9[b]
Bayer 29493 25%, EC	0.1	6	6	0	0	0	0	—	—	—
	0.4	16	18	2	2	2	2	—	—	—
Malathion 81%, EC	0.5	48	70	6	10	10	10	—	—	—
	2.0	54	60	0	0	0	0	—	—	—
Parathion 50%, EC	0.1	22	30	0	2	2	2	—	—	—
	0.4	92	100	38	44[b]	16	24[b]	2	2	—
DDT 25%, EC	0.5	20	34	2	4	4	4	—	—	—
	2.0	40	88	8	36[b]	12	22[b]	14	14[b]	2
Bayer 25141 25%, EC	0.2	34	60	2	6	6	6	—	—	—
	0.8	100	100	100	100[b]	90	92[b]	42	96[b]	36[c]
Control	—	4	4	2	0	2	2	0	0	0

[a] Treated on August 13, 1960.
[b] Fifty fresh fish replenished per plot at the time of or 24 hours prior to reading the mortality on long intervals of testing.
[c] Replenishment continued 24 hours prior to mortality readings taken 16, 26, 30, and 32 days after treatment with 76%, 42%, 30%, and 30% mortality, respectively.

FIG. 1.—Experimental mosquito breeding ponds constructed by the Kern Mosquito Abatement District where the toxic effects of insecticides on *Gambusia affinis* were studied. The fish were confined in the screen cages seen in the ponds.

clined and ranged from 63° to 82° F. during the day.

Prior to application of the materials the ponds were flooded with canal water. The water depth in the ponds ranged from 4 to 8 inches, causing some variation in the concentration of the materials in the water. However, this variation was insignificant insofar as the initial and residual toxicity of the materials against the fish was concerned. The treatments were assigned at random to the ponds.

Emulsifiable concentrate formulations of the materials as prepared by commercial formulators or basic manufacturers of the compounds were employed in preparing dilute aqueous sprays. The sprays were applied with 3-gallon hand sprayers using 20 to 30 lbs./sq. inch pressure and No. 8002 TeeJet nozzles. The sprays were applied at the rate of 0.5 gallon per plot or 8 gallons per acre.

The fish to be exposed in the treated water in the ponds were collected in rice fields and in sloughs located in the

Table 2.—Toxicity of insecticides to *Gambusia affinis* in the field.[a]

Material and Formulation	Toxicant (lb./acre)	Per Cent Mortality of Fish at Indicated Intervals After Treatment								
		Hours			Days					
		24[b]	48	72	5	6	9[b]	13[b]	20[b]	21
Bayer 25198 50%, EC	0.1	4	6	6	7	—	—	—	—	—
	0.4	8	8	8	9	—	—	—	—	—
E.N. 18133 50%, EC	0.1	100[b]	60[b]	42	—[c]	—	—	—	—	—
	0.4	100[b]	100[b]	100	*	100[d]	80	76	0[d]	0
AC-5727 15%, EC	0.2	2	2	2	2	—	—	—	—	—
	0.8	20	22[b]	0	0	—	—	—	—	—
GC-3583 50%, EC	0.5	100[b]	100[b]	100	*	100[d]	100	100	62[d]	92
	2.0	100[b]	100[b]	100	*	100[d]	100	100	100[d]	100
Dowco 139 25%, EC	0.1	2	2	2	2	—	—	—	—	—
	0.4	0	0	0	0	—	—	—	—	—
Control	—	2	2	2	2	—	—	—	—	—
	—	4	4	4	6	—	—	—	—	—

[a] Treated on August 24, 1960.
[b] Fifty fish replenished per plot at the time of or 24 hours prior to reading the mortality on long intervals of testing.
[c] Pond dried.
[d] Ponds reflooded 24 hours earlier.

Table 3.—Toxicity of insecticides to *Gambusia affinis* in the field.[a]

Material and Formulation	Dosage, lbs. Toxicant Acre	Per Cent Mortality of Fish at Indicated Intervals After Treatment				
		24 Hrs.	48 Hrs.[b]	72 Hrs.	8 Days[b]	15 Days[b]
SD-4402 15%, EC	0.1	100[b]	100	90	0	—
	0.4	100[b]	100	100	34	—
Methyl Trithion 50%, EC	0.5	14	60	40	0	—
	2.0	76	86	50	0	—
Bayer 38156 50%, EC	0.1	100[b]	72	18	0	—
	0.4	100[b]	100	100	6	—
GC-4072 50%, EC	0.2	100[b]	100	100	0	0
	0.8	100[b]	100	100	98	30
K-6882 25%, EC	0.2	0	0	0	0	—
	0.8	0	0	0	0	—
Control	—	0	0	0	0	0
	—	0	0	0	0	0

[a] Treated on September 12, 1950.
[b] Fifty fresh fish added per plot on these dates or 24 hours prior to the reading of mortality on long intervals of testing.

Table 4.—Toxicity of insecticides to *Gambusia affinis* in the field.[a]

Material and Formulation	Toxicant (lb./acre)	Per Cent Mortality of Fish at Indicated Intervals After Treatment					
		24 Hrs.[b]	48 Hrs.[b]	72 Hrs.[b]	7 Days[b]	14 Days[b]	21 Days[b]
E.N. 18133 50%, EC	0.2	100	100	94	54	16	0
	0.8	100	100	100	100	94	88[c]
GC-3583 50%, EC	0.2	98	100	0	0	—	—
	0.8	100	100	100	100	100	48[d]
GC-4072 50%, EC	0.1	10	12	0	0	—	—
	0.4	100	100	100	48[e]	0	0
SD-4402 18%, EC	0.2	100	100	48	0	0	—
	0.8	100	100	100	76	22	0
Dekafos	0.8	48	0	0	0	—	—
	1.6	90	46	0	0	—	—
Control	—	0	2	0	0	0	0
	—	0	0	2	0	0	0

[a] Treated on October 5, 1960. Average depth of water 6 to 8 inches.
[b] Fifty fresh fish replenished per plot at the time of or 24 hours prior to reading the mortality on long intervals of testing.
[c] 24-hour mortality was 50% and 0%, 28 days and 35 days after treatment, respectively.
[d] No mortality 28 days after treatment.
[e] The remaining fish looked sick.

vicinity of the ponds. A total number of 50 fish (mixed population), 25 fish per cage, were exposed in each pond at each testing period. The cage was made by stapling galvanized window screen on wooden frames. The cages were 15 inches high and 1 square foot in cross section (fig. 1). To avoid having the fish jump out, the cages were provided with loose lids of hardware cloth.

Fish were exposed in the treated water right after treatment and subsequently at various intervals. Fish mortality was recorded 24 hours after exposure, and if the mortality was low at the 24-hour interval, the cumulative mortality was read 48 or 72 hours or longer after exposure. In instances where the mortality was high, fresh fish were exposed and the mortality was read 24 hours or longer after the exposure. In determining the residual activity of certain long-lasting materials, fresh batches of fish were exposed 24 hours prior to the time of reading the fish mortality.

Results and Discussion.—Each material was evaluated for its toxicity against the fish at two to four dosage levels. The lower dosages were those of standard or common materials which are currently used for short-term mosquito control in California. For experimental compounds the lower dosages were based on the effectiveness of these materials against mosquito larvae in the field (Mulla et al. 1960, 1961). The lower dosages administered are the probable maximum rates which might be used when the materials become available and are approved for mosquito control in the future.

The higher dosages were arbitrarily set by quadrupling the lower dosages of each material, except Dekafos, where the lower dosage was only doubled. These high dosages are considered to be the probable maximum dosages employed for long-lasting mosquito larval control, or agricultural pest control programs in areas where intensive mosquito breeding occurs. Both the lower and the higher rates were deliberately set slightly on the maximum side to afford some safety margin.

Malathion, parathion and DDT are currently employed in larviciding programs for mosquito control in California. The rates of application usually vary from region to region, but most of the applications are made at the lower rates as indicated in table 1. At these lower rates malathion, parathion and DDT caused appreciable mortality of the fish 24 hours after treatment (table 1). The cumulative mortality slightly increased 48 hours after treatment. Malathion resulted in greater mortality of the fish than parathion or DDT. The last two materials were similar in their toxicity to the fish. All three materials at the lower dosages resulted in no appreciable mortality of the fish 72 hours after treatment, thus indicating the short residual toxicity of these materials at the lower dosages.

At the higher dosages parathion proved to be more toxic to the fish than malathion and DDT up to 6 days after treatment. The overall toxicity of DDT at the higher dosage was greater than the toxicity of malathion. The latter material, even at the higher dosage, produced no fish mortality 72 hours after treatment. Parathion and DDT at the higher dosage levels produced high mortality 48 hours after treatment, but the fish mortality declined at subsequent intervals.

At both rates Bayer 29493 (Baytex) produced a low cumulative mortality of the fish 48 hours after treatment. Beyond 48 hours this material produced no appreciable fish mortality at either of the dosages, indicating this material to be safe for use in situations where high fish mortality is undesirable.

Bayer 25141 produced a cumulative 60% mortality of the fish at the 0.2 lb./acre dosage level 48 hours after treatment (see table 1). After this period the material produced no appreciable mortality of the fish. At the higher dosage (0.8 lb./acre) this material proved highly toxic to the fish and its residual activity lasted for more than 30 days (table 1).

Bayer 25198 and Dowco 139 (Zectran) were essentially nontoxic to the fish at the rates used (table 2). Further observations on the latter material when it was applied at 1.0 lb./acre (an effective larviciding rate for this material), proved it to be nontoxic to the fish. Hercules Powder Company Compound AC-5727 was nontoxic at 0.2 lb./acre, but manifested some initial toxicity where it was applied at the rate of 0.8 lb./acre (table 2). At the higher rate this material showed no toxicity to the fish beyond 48 hours after treatment.

American Cyanamid Experimental Nematocide 18133 at 0.1 and 0.4 lb./acre and General Chemicals Compound

GC-3583 at 0.5 and 2.0 lb./acre proved highly toxic to the fish (table 2). E.N. 18133 at the lower dosage (0.1 lb./acre) resulted in complete kill of the fish 24 hours after treatment, but its toxicity gradually declined at subsequent testing intervals. At the higher dosage (0.4 lb./acre) this material proved to be highly toxic to the fish up to approximately 2 weeks after treatment. GC-3583 resulted in almost complete mortality of the fish at both 0.5 lb./acre and 2.0 lbs./acre up to 3 weeks after treatment. The toxicity of this material to fish in this experiment was not studied beyond the 3-week period after treatment.

Shell Development Company Compound SD-4402 proved highly toxic to the fish at the outset, at both 0.1 and 0.4 lb./acre rates (table 3). The toxicity at both rates measured 72 hours after treatment was very high. The material at the lower dosage proved nontoxic to the fish 8 days after treatment, but at the higher dosage manifested moderate toxicity 8 days after treatment.

Methyl Trithion at 0.5 and 2.0 lbs./acre killed 60% and 80% of the fish, respectively, 48 hours after treatment (table 3). The toxicity of this material, however, declined and lower kill of fish was obtained 72 hours after treatment. Eight days after treatment this material proved nontoxic at both dosages.

Bayer 38156 proved toxic to the fish initially at both 0.1 and 0.4 lb./acre rates. The toxicity of this material at the lower dosage, however, declined rapidly, and low fish mortality was obtained 72 hours after treatment. At the higher dosage, this material resulted in complete kill of the fish up to 3 days after treatment. Negligible mortality of fish was obtained at this dosage 8 days after treatment.

General Chemicals Compound GC-4072 proved highly toxic to the fish. At 0.2 and 0.8 lb./acre dosages, the material resulted in complete kill of the three successive batches of fish exposed right after, 24 and 48 hours after treatment (table 3). The lower dosage of this material produced no fish mortality 8 days after treatment. The mortality produced by the higher dosage 8 and 15 days after treatment was 98% and 30%, respectively.

Dow Chemical Company Compound K-6882 proved nontoxic to the fish, at both 0.2 and 0.8 lb./acre rates (table 3). This material is considered to be safe for use in situations where *Gambusia affinis* populations are thriving.

All materials (with the exception of Bayer 25141) that manifested residual activity against the fish were retested for their toxicity to the fish at lower or higher dosages than those indicated in tables 2 and 3. On account of the stability of GC-3583 and GC-4072, long-lasting control of mosquito larvae was obtained with the dosages indicated earlier (see tables 2 and 3). It was deemed possible that these materials could be used at slightly lower dosages for effective larval control. Therefore, these two materials were retested at 0.2 and 0.8 lb./acre and 0.1 and 0.4 lb./acre, respectively.

E.N. 18133 and SD-4402 were re-evaluated for their toxicity to the fish at slightly higher dosages than those indicated in tables 2 and 3. In light of recent information the lowest dosages (at which these two materials were evaluated) are considered to be the effective larviciding dosages. These lowest dosages were increased for the purpose of obtaining information on the activity of these materials against the fish at these higher larviciding dosages which might be employed for obtaining residual control. Dekafos, a new material, was used at two dosages (0.8 and 1.6 lb./acre) and the material was concurrently determined to have low activity against the mosquito larvae. Even at the higher dosage the material did not yield complete kill of the larvae (Mulla et al. 1961).

At the revised higher dosages, all materials proved highly toxic to the fish initially (table 4). The lower dosages of all materials except Dekafos also proved lethal to the fish at the outset. Dekafos at neither dosage caused any fish mortality 72 hours after treatment. The lower dosage of E.N. 18133 (0.2 lb./acre) was more residual than the lower dosages of the other materials listed in table 4. The lower dosages of GC-3583 and GC-4072 did not cause fish mortality from 48 to 72 hours after treatment. The lower dosage of SD-4402 (0.2 lb./acre), however, produced considerable mortality after this interval. At the higher dosages (0.8 lb./acre), E.N. 18133 and GC-3583 were found to have the most residual toxicity against the fish. SD-4402 at 0.8 lb./acre manifested residual activity against the fish 2 weeks after treatment. GC-4072 at 0.4 lb./acre proved to be toxic to the fish for slightly more than a week. This material at this dosage produced no fish mortality 2 weeks after treatment. The results obtained with the application of the revised dosages of E.N. 18133, GC-3583, GC-4072, and SD-4402 complement those presented in tables 2 and 3.

The degree of toxicity or safety of moderately toxic materials to the fish will depend to a great extent on the frequency of their application in mosquito or agricultural pest control programs (Mulla 1961). Repeated applications of materials having intermediate toxicity to the fish will probably result in noticeable reduction of the fish populations even at the mosquito larviciding rates. Materials of this category are malathion, parathion, DDT, Bayer 25141, and Methyl Trithion. When used routinely for mosquito control these materials will suppress fish populations markedly. On the other hand, frequent applications of safe materials such as Bayer 25198, AC-5727, Zectran (Dowco 139), Dow K-6882, and Bayer 29493 for the control of mosquitoes and agricultural pests would be seemingly compatible with maintaining high populations of the fish. These materials were also observed to be relatively safe on predaceous insects (Mulla et al. 1960, 1961). AC-5727 and Bayer 29493 when used repeatedly at agricultural pest control dosages would cause some reduction in fish populations but frequent usage of these materials is not foreseen at least in the near future.

The highly toxic materials will wipe out fish populations even if applied once. Some of these materials will eliminate fish at the lower rates. The higher rates of the residual materials, namely Bayer 25141, GC-3583, GC-4072, SD-4402, and E.N. 18133, will preclude the reintroduction of fish into the treated area from 1 to 4 weeks.

The residual materials will also yield long-lasting control of mosquito larvae. These materials can be profitably used in locations where mosquito fish play a minor role in larval reduction. These materials, although highly toxic to the fish, could play a major role in future mosquito control programs in California, until and unless other undesirable features of these control agents are revealed.

The information gathered herein clearly presents a

sound basis for the integration of chemical and biological control of mosquitoes. The selection of an appropriate material will result in more efficient and economic control of pest and vector mosquito species. Selection of suitable and desirable materials will be dictated by local conditions and the type of mosquito control technology in operation. Agricultural pest control operators should be made aware of the mosquito problems. Coordinated approach toward crop pest control and mosquito control, if pursued, would undoubtedly benefit both mosquito abatement and agricultural pest control agencies.

CHEMICAL NAMES OF PROPRIETARY MATERIALS

American Cyanamid E.N. 18133.—*O,O*-diethyl *O*-2-pyrazinyl phosphorothioate.

AC-5727.—*m*-isopropylphenyl *N*-methylcarbamate.

Bayer 25141.—*O,O*-diethyl *O*-*p*-(methylsulfinylphenyl)phosphorothioate.

Bayer 25198.—*O,O*-dimethyl *O*-*p*-(methylsulfinylphenyl)phosphorothioate.

Bayer 29493 (*Baytex®*).—*O,O*-dimethyl *O*-[4-(methylthio)-*m*-tolyl] phosphorothioate.

Bayer 38156.—*O*-ethyl *S*-*p*-methylphenyl ethylphosphonodithioate.

Dekafos®.—3-pentadecylphenol *O,O*-diethylthiono phosphate and related compounds.

Zectran® (*Dowco 139*).—4-dimethylamino-3,5-xylyl methylcarbamate.

Dow K-6882.—*O*-ethyl *O*-(2,4,5-trichlorophenyl) methyl phosphoroamidothioate.

GC-3583.—diethyl-1-(2,5-dichlorophenyl)-2-chlorovinyl phosphate.

GC-4072.—diethyl-1-(2,4-dichlorophenyl)-2-chlorovinyl phosphate.

Methyl Trithion®.—*O,O*-dimethyl S-[(p-chlorophenylthio) methyl] phosphorodithioate.

SD-4402.—1,3,4,5,6,7,8,8-octachloro 3a,4,7,7a-tetrahydro-4,7-methanophthalan.

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Synergism of Carbamate Insecticides Against Cabbage Looper and Beet Armyworm Larvae[1]

H. H. Shorey,[2] *University of California Citrus Experiment Station, Riverside*

Abstract

Piperonyl butoxide and Monsanto 16226 (bis (2,3,3,3-tetrachloropropyl) ether) were tested as synergists in combination with Sevin® (1-naphthyl *N*-methylcarbamate), Hercules 5727 (*m*-isopropylphenyl *N*-methylcarbamate) and Zectran® (4-dimethylamino-3,5-xylyl *N*-methylcarbamate). In 1:1 mixtures with piperonyl butoxide, tested against larvae of the cabbage looper, *Trichoplusia ni* (Hübner), Sevin was potentiated 10.3-fold, Hercules 5727, 2.6-fold, and Zectran, not at all. Against larvae of the beet armyworm, *Spodoptera exigua* (Hübner), Sevin and Zectran were potentiated 4.4-fold and 1.2-fold, respectively. An increase in the ratio of piperonyl butoxide to carbamate generally provided a corresponding increase of potentiation against the cabbage looper. Monsanto 16226, with a relatively high innate toxicity against both species, was not considered as efficient a synergist as piperonyl butoxide.

Following the demonstration by Moorefield (1958) that the carbamate insecticides could be potentiated by a variety of methylenedioxyphenyl compounds, considerable interest has been exhibited by several toxicology laboratories in exploring this line of research. Two possibilities indicated by such studies have considerable importance in work relating to the control of economic pest insects. One possibility, which has been demonstrated with the house fly, *Musca domestica* L., by Moorefield (1960), Georghiou & Metcalf (1961a), and Georghiou et al. (1961), is that insects having attained a resistance to carbamates may remain relatively susceptible to carbamate-synergist mixtures. This susceptibility is generally considered to result from a blocking of the carbamate-detoxication mechanisms by the synergist. In addition, Moorefield (1960) and Georghiou et al. (1961) have found only slight increases in tolerance among house flies maintained under selection pressure from carbamate-synergist mixtures.

The second possibility, and that with which the present study is concerned, is that a synergist might sufficiently potentiate a candidate carbamate insecticide to broaden the scope of its effectiveness against insect pests. Metcalf et al. (1960) and Moorefield (1960) have observed that many carbamates tend to be highly specific in insecticidal performance. Sevin® (1-naphthyl *N*-methylcarbamate), which has reached a broad commercial usage, has a very high insecticidal efficiency against certain insect species, and a relatively low efficiency against others. Within the order Lepidoptera, Sevin has been noted for its effectiveness against the corn earworm, *Heliothis zea* (Boddie); it is considered of marginal commercial value in southern California, however, against the cabbage looper, *Trichoplusia ni* (Hübner), and the beet armyworm, *Spodoptera exigua* (Hübner).

Methods and Materials.—The carbamate insecticides investigated were Zectran® (4-dimethylamino-3,5-xylyl *N*-methylcarbamate), Hercules 5727 (*m*-isopropylphenyl *N*-methylcarbamate), and Sevin. Piperonyl butoxide and Monsanto 16226 (bis(2,3,3,3-tetrachloropropyl)ether) were selected as synergists. Monsanto 16226 was chosen because it does not belong to the familiar class of methylenedioxyphenyl pyrethrins synergists. It has been used as a synergist for carbamate insecticides against house flies by Georghiou & Metcalf (1961b).

A cabbage looper colony was established from individuals collected in various areas of southern California; beet armyworms were colonized from several locations in Imperial County, California. The colonies were maintained in a greenhouse at 80° F. Cabbage looper larvae were confined beneath 1×1×1-foot cages on flats of beans, while the armyworms were reared on lettuce leaves in ½-pint food cartons. Both colonies were reared through at least one complete generation before testing was begun.

Acetone solutions of the carbamates, synergists, and carbamate-synergist mixtures were applied topically with a micrometer-driven syringe delivering 1 μl. per larva. The drop was applied dorsally in the vicinity of the first abdominal segment. Third-instar larvae of both species, averaging approximately 6 days old, were chosen for treatment. Weight was recognized as a potentially large source of experimental variation; therefore, only early third-instar beet armyworm larvae were used.

Both cabbage looper and beet armyworm larvae were treated without anesthetization. Early attempts at anesthetization were unsatisfactory; the larvae flattened against their substrate, increasing the probability of some loss of the applied solution. Application of the acetone solutions to unanesthetized beet armyworm larvae was not difficult because the larvae tended to be sluggish. But the applied drop spread over large areas of the relatively glabrous cuticle, with a slight loss to the substrate in some cases. This spreading was particularly noticeable in treatments containing a high concentration of piperonyl butoxide. Very little spreading of the solutions occurred on cabbage looper larvae; the drop was typically maintained by setae on a confined area of the cuticle until it evaporated.

The unanesthetized cabbage looper larvae, however, tended to be very active. In the technique finally evolved, a breath of air was used immediately before treatment to cause the larvae to freeze in a characteristic S-shaped position with the anterior portion of the body elevated. Application was effected easily during the few seconds that this position was maintained.

During application the larvae were supported on leaves: lettuce for beet armyworms and either lettuce or beans for cabbage loopers. Following treatment, the larvae and leaves were placed in ½-pint food cartons and were held for 24 hours at 80° F. Five larvae in a single

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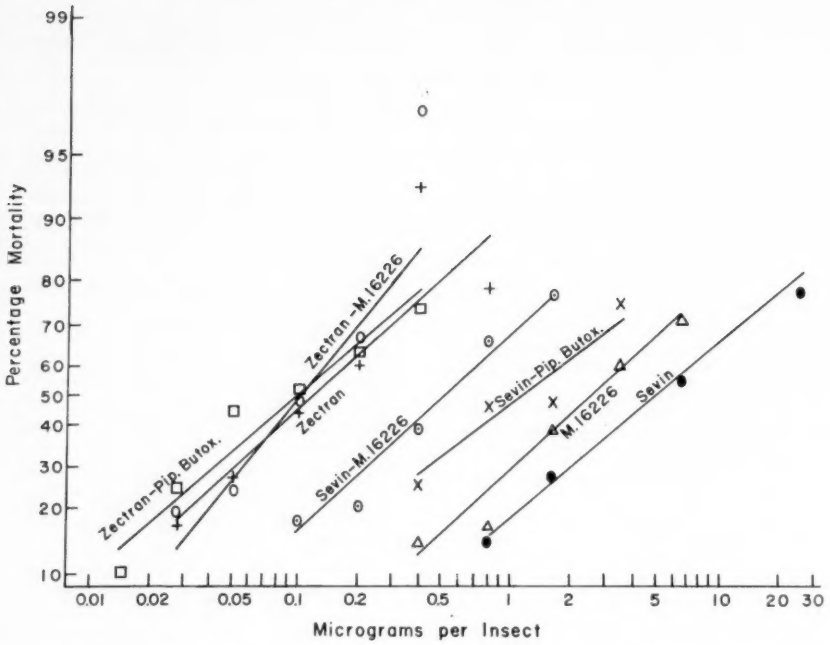

FIG. 1.—Susceptibility of beet armyworm larvae to Sevin alone (●) and in 1:1 mixtures with piperonyl butoxide (×) and Monsanto 16226 (⊙), to Zectran alone (+) and in 1:1 mixtures with piperonyl butoxide (□) and Monsanto 16226 (○), and to Monsanto 16226 alone (△). Each point represents percentage mortality on a logit scale plotted against the micrograms of insecticide per insect in the case of a carbamate or synergist alone, or against the micrograms of carbamate per insect in a mixture.

carton constituted one replicate. Ten replicates for each concentration of each carbamate, synergist, or carbamate-synergist mixture tested against the cabbage looper were set up over a period of several days; six for each were tested against the beet armyworm.

Larvae exhibiting a lack of coordinated movement when prodded were considered dead. Check mortality among cabbage looper larvae treated with 1 μl. of acetone alone averaged 3% and was rectified in the summarization of data by the use of Abbott's formula (Abbott 1925). No check mortality occurred during the beet armyworm tests.

RESULTS AND DISCUSSION.—Figure 1 presents curves resulting from topical applications to beet armyworm larvae of 1:1 mixtures of piperonyl butoxide or Monsanto 16226 with Zectran or Sevin. Similar curves recording the effects upon cabbage looper larvae of mixtures of the two synergists with Sevin are given in figure 2, and with Zectran and Hercules 5727 are given in figure 3. The dosage-mortality lines were fitted by the method of logit analysis (Berkson 1953).

LD_{50}'s determined from the dosage-mortality lines for carbamates, synergists, and their mixtures are presented in tables 1 and 2 for beet armyworms and cabbage loopers, respectively. Synergism ratios in the tables, if greater than 1, indicate the probability of synergism. These ratios were computed at the LD_{50} level as an index of the effectiveness of the mixture in excess of the effectiveness which might be expected as a result of similar joint action of the individual components (Wadley 1945).

Monsanto 16226 was an inefficient synergist at 1:1 ratios for the carbamates tested; the maximum potentia-

Table 1.—Results of dosage-mortality studies with carbamates, synergists, and their mixtures topically applied to beet armyworm larvae.

MATERIALS	A	B	
	LD_{50} (μg.)[a]	LD_{50} Mixture in Carbamate Equivalents[b]	SYNERGISM RATIO[c]
Zectran	0.13		
Sevin	5.83		
Monsanto 16226	2.46		
Piperonyl butoxide	500		
Zectran: Monsanto 16226 (1:1)	0.11	0.12	1.1
Zectran: Pip. but. (1:1)	0.11	0.11	1.2
Sevin: Monsanto 16226 (1:1)	0.54	1.82	3.2
Sevin: Pip. but. (1:1)	1.34	1.34	4.4

[a] LD_{50}'s for the mixtures of carbamates with synergists are listed in terms of the μg. of carbamate used in each mixture.

[b] These LD_{50}'s are obtained from percentage mortalities plotted against each concentration of the mixture computed in terms of μg. of carbamate plus (μg. of synergist × equivalence factor) as described by Wadley (1945).

[c] Ratio of LD_{50} for carbamate alone (column A) divided by LD_{50} in carbamate equivalents (column B) for the mixture of that carbamate plus a synergist. If this ratio approximates 1, synergism is not indicated; if the ratio exceeds 1, synergism can be suspected.

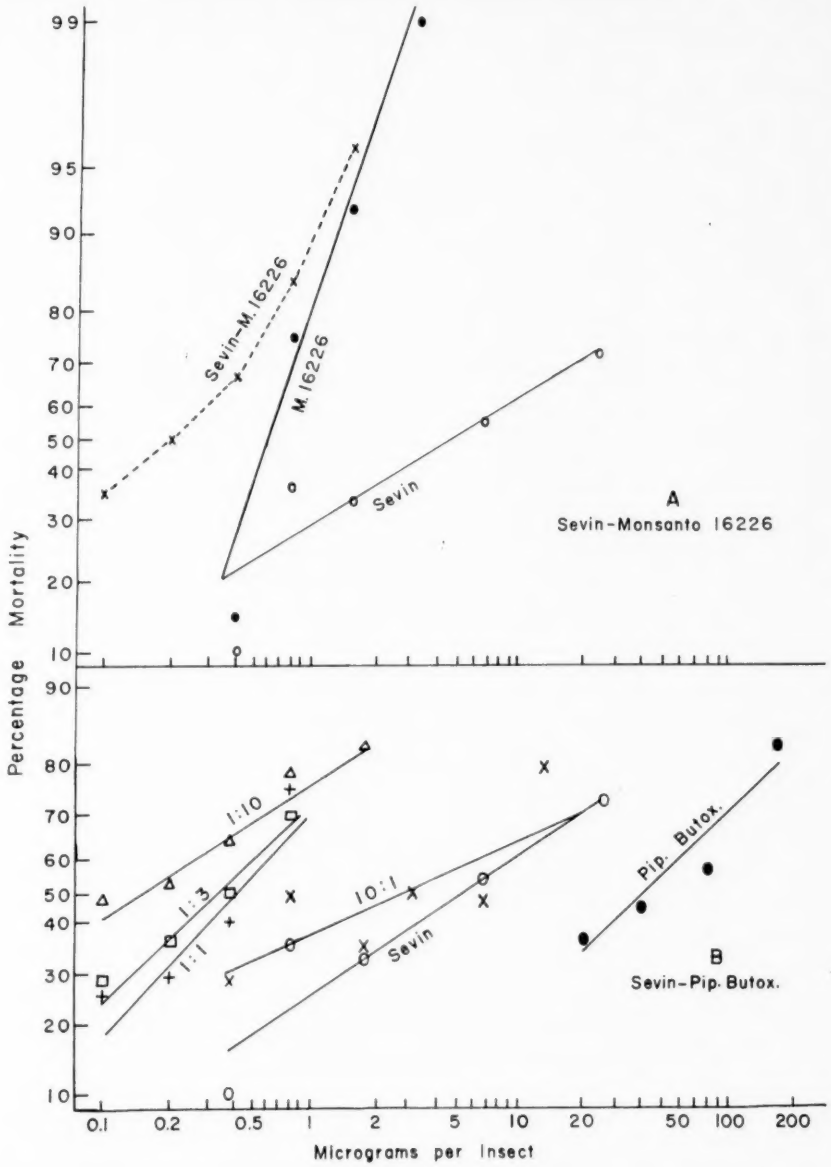

FIG. 2.—Susceptibility of cabbage looper larvae to Sevin (○); (A) to Monsanto 16226 alone (●) and in a 1:1 mixture with Sevin (×); (B) to 1:10 (△), 1:3 (□), 1:1 (+), and 10:1 (×) mixtures of Sevin with piperonyl butoxide, and to piperonyl butoxide alone (●). Each point represents percentage mortality on a logit scale plotted against the micrograms of insecticide per insect in the case of a carbamate or synergist alone, or against the micrograms of carbamate per insect in a mixture.

tion produced, with Sevin applied to beet armyworm larvae, was 3.2-fold. This low degree of activation may be due partially to the relatively high toxicity of Monsanto 16226 which was more toxic to both species than Sevin alone. The mortality directly attributable to Monsanto 16226 could have partially masked that caused by potentiation of Sevin. For the cabbage looper (fig. 2), although there is some indication of possible potentiation of Sevin by Monsanto 16226 at low concentrations, no such activity can be seen at higher concentrations because the dosage-mortality line depicting their 1:1 mixture merges upon the line for Monsanto 16226 alone.

Varying degrees of potentiation of the carbamates by piperonyl butoxide were noted for cabbage looper larvae

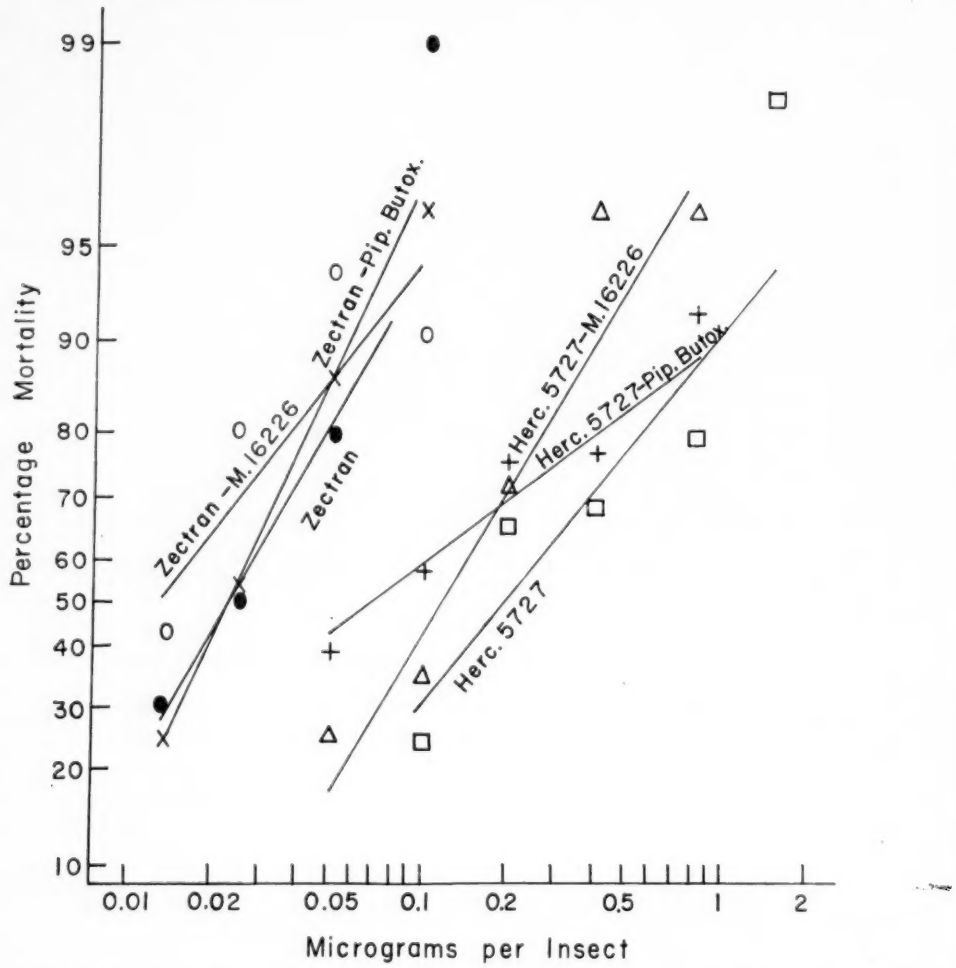

FIG. 3.—Susceptibility of cabbage looper larvae to Zectran alone (●) and in 1:1 mixtures with piperonyl butoxide (×) and Monsant[9] 16226 (○), and Hercules 5727 alone (□) and in 1:1 mixtures with piperonyl butoxide (+) and Monsanto 16226 (△). Each point represents percentage mortality on a logit scale plotted against the micrograms of insecticide per insect in the case of a carbamate or synergist alone, or against the micrograms of carbamate per insect in a mixture.

(table 2). The synergism ratios found among the three carbamates are in accord with observations by Metcalf et al. (1960), who found that the degree of carbamate synergism against house flies appeared to be inversely correlated with the innate toxicity of the compound. Sevin, with an LD_{50} of 4.76 μg., was synergized 10.3-fold at the LD_{50} level by piperonyl butoxide at a 1:1 ratio. Hercules 5727 and Zectran, with LD_{50}'s of 0.19 and 0.022 μg., respectively, were synergized 2.6-fold and not at all, respectively. A similar trend was observed with beet armyworm larvae (table 1).

Although Sevin is relatively highly synergized by piperonyl butoxide against the cabbage looper, the dosage-mortality lines for the mixtures are not markedly steeper than the line for Sevin alone (fig. 2). The shallow slope obtained with Sevin alone helps to explain why this insecticide has given no more than fair control of cabbage looper larvae in the field in southern California (Hall et al. 1961). A similarly shallow slope for Sevin-piperonyl butoxide mixtures may partially negate the 10- to 20-fold potentiation owing to synergism by causing an insufficient increase in mortality over an equivalent dosage of Sevin alone to be of economic consequence. Recent field research has indicated that this is the case; 1:1 mixtures of Sevin and piperonyl butoxide did not give a marked improvement in control of cabbage looper and beet armyworm larvae over that provided by Sevin alone (Shorey et al. 1961).

Cabbage looper mortalities resulting from the topical application of a fixed dosage of each carbamate mixed in various ratios with each synergist are summarized in table 3. Thirty to 50 larvae were tested for each com-

Table 2.—Results of dosage-mortality studies with carbamates, synergists, and their mixtures topically applied to cabbage looper larvae.

MATERIALS	A	B	
	LD_{50} (μg.)[a]	LD_{50} in Mixture in Carbamate Equivalents[b]	SYNERGISM RATIO[c]
Zectran	0.022		
Hercules 5727	0.19		
Sevin	4.76		
Monsanto 16226	0.64		
Piperonyl butoxide	42.7		
Zectran: Monsanto 16226 (1:1	0.012	0.012	1.8
Zectran: Piperonyl butoxide (1:1)	0.022	0.022	1.0
Hercules 5727: Monsanto 16226 (1:1)	0.11	0.014	1.4
Hercules 5727: Piperonyl butoxide (1:1)	0.073	0.073	2.6
Sevin: Monsanto 16226 (1:1)	0.20[d]		
Sevin: Piperonyl butoxide (10:1)	2.86	2.89	1.6
Sevin: Piperonyl butoxide (1:1)	0.41	0.46	10.3
Sevin: Piperonyl butoxide (1:3)	0.35	0.47	10.1
Sevin: Piperonyl butoxide (1:10)	0.12	0.27	17.6

[a] See footnote [a], table 1.
[b] See footnote [b], table 1.
[c] See footnote [c], table 1.
[d] An approximate figure read from a curved line (fig. 2).

Table 3.—Effect of varying ratios of synergist to carbamate on mortality of cabbage looper larvae.

CARBAMATE	μg./ LARVA	SYNERGIST CARBAMATE RATIO	PERCENTAGE MORTALITY			
			Piperonyl Butoxide		Monsanto 16226	
			Mixture	Expected from Synergist Alone[a]	Mixture	Expected from Synergist Alone[a]
Sevin	0.4	0:1	9			
		1:10	28	0	42	1
		1:3	—	—	50	1
		1:1	40	2	48	19
		3:1	49	4	84	87
		10:1	62	12	94	99
Hercules 5727	0.1	0:1	16			
		1:10	3	0	28	0
		1:3	27	0	32	0
		1:1	23	1	42	1
		3:1	43	1	32	10
		10:1	57	4	76	78
Zectran	0.02	0:1	34			
		1:10	32	0	20	0
		1:3	30	0	42	0
		1:1	40	0	29	0
		3:1	43	0	33	1
		10:1	50	3	40	1

[a] Expected mortalities were read from the curves for piper onyl butoxide o Monsanto 16226 alone (fig. 2).

bination. An increase in the ratio of piperonyl butoxide to carbamate from 1:10 to 10:1 appeared to provide a corresponding increase in carbamate potentiation. This increase is marked for Sevin and for Hercules 5727, and less distinct for Zectran. Increases in carbamate potentiation resulting from increases in the ratio of Monsanto 16226 were slight; high mortalities caused by mixtures containing high proportions of Monsanto 16226 can be attributed directly to the toxic action of the synergist.

Zectran, although not appreciably synergized by piperonyl butoxide or Monsanto 16226, appears to be very effective when used alone against cabbage looper and beet armyworm larvae. Georghiou & Metcalf (1961c) and Shorey et al. (1961) have found that it is highly insecticidal to a variety of lepidopterous larvae. Zectran is very rapid in action; all larvae ultimately counted as dead were already dead or moribund within one-half hour after application. The area of the larval body to which Zectran was applied typically became darkened and constricted within a few minutes; usually the whole larva was found darkened and shriveled at the end of 24 hours. In contrast, larvae killed by Sevin maintained their original identity of color and form during the 24-hour holding period.

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Corn Earworm and European Corn Borer Resistance in Sweet Corn Inbred Lines[1]

W. D. Guthrie and E. V. Walter, *Entomology Research Division, Agric. Res. Serv., U.S.D.A.*[2]

Abstract

Evaluation of inbred lines of sweet corn for resistance to ear infestation by the corn earworm (*Heliothis zea* (Boddie)) and resistance to leaf feeding by the European corn borer (*Ostrinia nubilalis* (Hübner)) was made during a 5-year period. Some lines were found to be resistant to both species of insect. Origin of most of the inbreds is given. No consistent correlation could be shown between resistance to the corn earworm and resistance to the corn borer although many lines were resistant to both.

Development of sweet corn inbred lines resistant to the corn earworm (*Heliothis zea* (Boddie)) has been in progress for 20 years. Over a period of years many inbred lines of sweet corn have also been evaluated and selected for resistance to the European corn borer (*Ostrinia nubilalis* (Hübner)).

The objective of the investigations reported herein was to locate earworm and corn borer resistance germ plasm in the same inbred lines, and to assemble this information for plant breeders and entomologists interested in sweet corn breeding programs. The lines discussed were selected and developed by the junior author who has had a significant role in the earworm resistance program in sweet corn.

Methods.—Evaluations for resistance to both insect species were made during 1954, '55, '57, '59, and '60. Duplicate plantings were made of all lines, one at Lafayette, Indiana and one at Wooster, Ohio. Evaluations for earworm resistance were made at Lafayette and those for the corn borer at Wooster. Earworm-resistance evaluations were also made at Wooster in 1959.

At Lafayette each line was planted in single-row plots of 25 plants. During the early silking stage of plant development the ears of 12 to 15 plants in each plot were artificially infested with three newly hatched corn earworm larvae to supplement the natural infestation. Artificial infestation procedures with corn earworm larvae were reported by Blanchard et al. (1942).

A grading system described by Walter (1948) was used to evaluate corn earworm resistance. All artificially infested ears from each plot were graded at harvest in classes 0 to 5 according to the amount of feeding. Each class was determined as follows: Class 0, no injury; class 1, tip injury; class 2, kernels destroyed to one-half inch below the tip; class 3, kernels destroyed to 1¼ inches below the tip; class 4, kernels destroyed to 2½ inches below the tip; and class 5, kernel destruction exceeding 2½ inches below the tip. The percentage of ears in each class for each line was then determined. Many tests during the past several years have shown that the number of average-sized kernels destroyed for each of the six classes was, respectively, 0, 1, 10, 20, 30, and 40. The sum of the percentages of ears falling in each class for each inbred multiplied by the kernel factor and divided by 100 gives a close approximation of the area of an equivalent number of average-sized kernels. For example, if 15 or 60% of the ears from a 25-ear sample were in class 1, 1 kernel×60 =60; 4 or 16% of the ears were class 2, 10 kernels×16 =160; and 6 or 24% of the ears were class 3, 20 kernels ×24=480; then 60+160+480 equals 700 divided by 100=7, or the injury index number for the inbred. This injury index number is used in earworm resistance studies to compare one line with another. On the basis of this classification an index number of less than 12 is considered resistant, from 12 to 20 intermediate, and above 20 susceptible; for example, the well-known inbred P39 tests between 23 and 24 whereas I45 usually tests about 18. The injury index number for all inbreds recorded in table 1 was less than 12 in all tests.

Methods used in present-day corn borer resistance investigations in dent corn were reported by Dicke & Penny (1956) and Guthrie et al. (1960). The methods are similar for corn borer resistance investigations in sweet corn.

At Wooster each line was planted in a single-row plot of five 3-plant hills with 1- to 3-fold replication. Six to 10 plants in each plot were artificially infested with 4 to 6 egg masses, or approximately 80 to 120 eggs, per plant during the mid-whorl stage of plant development. The infestation was made in two applications of 2 to 3 masses each spaced a few days apart.

A nine-class rating system described by Guthrie et al. (1960) was used for evaluating "whorl" leaf-feeding injury. The ratings were made on a plot basis 3 weeks after egg hatch. Ratings of 1 to 3 were considered resistant, 4 to 6 intermediate, and 7 to 9 highly susceptible. Many tests have shown this rating system to be a good criterion for determining relative corn borer infestation or for evaluating inbred lines for resistance to a first-brood infestation.

Results and Discussion.—Several sweet corn inbred lines were resistant to both the corn earworm and European corn borer (table 1). Only lines resistant to both species of insect were recorded although many more were evaluated. Two corn borer-susceptible lines were included for checks each year in the Wooster nursery, and two earworm-susceptible lines in the nursery at Lafayette; the checks are not shown, however, in the table. Since all lines listed were resistant to both insects in all tests, specific ratings for each line are not given. The rating system appropriate for each insect was used, however, in compiling the table. All lines were experimental inbreds in a late stage of inbreeding at the time of testing, although inbred 245 has now been released to commercial breeders.

During the first few years of earworm-resistance studies each inbred was designated by its pedigree, which was followed by an ear selection number for each generation. If a line was graded resistant for several generations, it was assigned a number followed in parentheses by the

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[2] In cooperation with Ohio Agricultural Experiment Station, Wooster, and Purdue Agricultural Experiment Station, Lafayette, Indiana.

Table 1.—Experimental sweet corn inbreds found resistant to both the corn earworm and European corn borer, 1954–60.

INBRED	ORIGIN OF INBRED[a]	NUMBER OF		DAYS TO 50% SILK
		Earworm Tests	Corn Borer Tests[b]	
10(13)1-3-2-1-2-1-2	Flourcorn×P39	13	*4	62
34(7)2-3-1	Flourcorn×Flourcorn×P39 OX	7	2	70
38(9)1Y-1-1-1-3	Calumet×Flourcorn	9	*3	74
40(6)1Y-1	Calumet×Flourcorn	6	4	67
41(6)2-1	Calumet×Flourcorn	6	6	62
42(10)1-1-1-2-1-1	Calumet×Flourcorn	10	7	72
214A(8)1-1-1-1-1-1	14N×Od3	8	2	58
214A(8)1-1-1-1-1-2	14N×Od3	8	*2	58
245(8)1-2Y-2-1-1-1-1	P39×81-1	8	6	77
266(11)1-2-1-2-1-1-1-1-1	Flourcorn×P39	11	1	69
287(12)2-1-1-1-1-1-1-1-1-1-2	81-1×Od3	12	1	83
322(6)3-2-1	Calumet×(P39×81-1)	6	2	70
332(6)1-1	Teosinte×sweet corn	6	*2	64
334(6)1-1	Teosinte×sweet corn	6	2	59
345(8)1-2-1-2-1	Teosinte×sweet corn	8	*5	69
347(8)1-1-1-1-2	Teosinte×sweet corn	8	3	66
350(8)2-1-1-1-1	Teosinte×sweet corn	8	*3	66
361(6)1-3	I11.16884×81-1	6	**4	77
374(6)2-3	(Ia2166A×Od3)×81-1	6	**4	66
377(6)1-1	(Flourcorn×P39)×Od3	6	4	74
380(6)2Y-3	I11.73C×166	6	3	70
381(8)2-3	I11.73C×166	8	1	69
382(8)1-3	P39×166	8	*3	75
384(6)1-3	(Teosinte×sweet corn)×166	6	*4	76
384(6)3-3	(Teosinte×sweet corn)×166	6	4	73
385(6)1-1	(Teosinte×sweet corn)×166	6	4	76
385(6)2-3	(Teosinte×sweet corn)×166	6	4	76
389(7)1-3	166×M119	7	3	75
392(6)3-3	M119×214A	6	4	68
393(6)1-3	M119×245	6	†4	69
396(6)1-1	M119×(Teosinte×sweet corn)	6	4	63
397(6)1-3	M119×(Teosinte×sweet corn)	6	†4	67
399(6)1-3	245×(Teosinte×sweet corn)	6	4	72
402(6)1-3		6	4	78
403(6)1-3		6	**4	74
406(6)1-3	245×C88	6	**4	74
408(6)1-3	245×(I11.107a×81-1)	6	4	71
411(5)2Y-3	214A×245	5	4	75
412(6)1-3	I11.151×245	6	4	69
413(6)1-3	I11.151×245	6	4	69
414(6)1-3	245×Ia5125	6	4	69
415(6)1-3	245×(Ia2166A×Od3)	6	4	73
415(6)2-3	245×(Ia2166A×Od3)	6	4	73
416(6)1-3		6	4	76
416(5)2		5	1	76
417(6)1-1	214A×(E13×Od2)	6	4	73
419(5)2		5	1	75
(245×175-51)(6)1-2-1-1-3		6	3	74
(14N×274(4)1-1-1)(3)1-1		3	*2	58
M118-8-2-4-3-2L1-1		7	2	79
M119-8-7-4-2-L1-2-3-3-1		10	2	70
I11.16884-61-53-T1-L1		2	2	63

[a] All inbreds except P39, Ill.73C, Ia5125, 81-1, 166, and 245 are experimental and have not been released. They are given to show the source of resistance.
[b] All inbred lines rated class 1 to 3 (in a 1 to 9 classification) with the exceptions indicated as follows: *One test rated class 4; **Two tests rated class 4; †One test rated class 5.

number of times it had been selfed; in subsequent generations the numbers represent the ears selected in successive generations. For example, 10(13)1-3-2-1-2-1-2 indicates that this was selection number 10, that it had been inbred 13 times, and the following figures indicate the particular ear selection in each generation after the pedigree was dropped and the number 10 substituted.

All inbreds listed in table 1 are midseason to late in maturity. All have in their ancestry some variety native to a tropical or subtropical area where corn has been subjected to a heavy earworm infestation for many years.

E. V. Walter (unpublished data) found several factors associated with earworm resistance in various sweet corn inbred lines, among which are the following: (1) Tight husks (depending largely on husk width) combined with closely placed kernel rows that restrict feeding by the larvae at the tip instead of allowing them to penetrate deeply along the surface of the ear; (2) a nutritional element that apparently limits the development of larvae; (3) an apparent silk "lethal factor" that causes a high mortality of larvae within a few hours after feeding or by the third-instar stage; and (4) larvae may leave the silks

of some lines by the third-instar stage, possibly because of a deficiency of some nutritional substance.

In reviewing corn earworm-resistance investigations in sweet and dent corn, from their inception through 1948, Painter (1951) listed several other factors that different investigators found to be possible mechanisms affecting the resistance of corn to the earworm.

Corn borer tests in these investigations were for relative resistance to a first-brood infestation. In discussing the biology of the first and second brood of the corn borer in the corn plant and its relation to resistance investigations, Dicke (1954) reported that tassel emergence occurs primarily after first-brood hatching in field corn and in midseason and late sweet corn. Factors that inhibit first-brood larval establishment and survival in resistant lines are operative against the early larval instars, which become established on the spirally rolled leaves in the whorl stage of plant development. Early planted, short-season sweet corn may be in the pollen-shedding stage of plant growth during part of the first-brood oviposition period. In this stage of growth young larvae become established on pollen in the florets, at the leaf axils where pollen has accumulated, and on ear parts. Thus, a much higher level of first-brood larval establishment and survival would be expected from an infestation originating during the pollen-shedding stage of plant development. A high level of establishment would also result from a second-brood infestation, which usually originates during some phase of plant flowering in the Corn Belt States.

Huber & Stringfield (1940, 1942) reported a correlation between the resistance of dent corn lines and hybrids to the corn leaf aphid (*Rhopalosiphum maidis* (Fitch)) and resistance to the European corn borer. Several Ohio dent corn lines and hybrids, which are used extensively at the present time and have corn borer resistance, are also resistant to the corn leaf aphid. Several of these lines were selected for aphid resistance during the early phase of inbreeding, when an infestation from a natural first-brood corn borer population was at a low level, and later found to be corn borer resistant also.

The sweet corn lines recorded in table 1 were selected primarily for earworm resistance and found later to be resistant to the corn borer. These data and the data reported by Huber & Stringfield (1940, 1942) suggest a possible method of evaluating inbred lines for resistance to two species of insects when either species is present. However, many inbred lines are resistant to one species of insect and susceptible to the other. Therefore, caution should be used in judging the resistance of an inbred to one insect as an index of its resistance to another species. No definite correlation could be shown between resistance to the corn borer and that to the corn earworm although many lines were resistant to both.

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The Relative Effectiveness of Two Synthetic Pyrethroids More Toxic to House Flies than Pyrethrins in Kerosene Sprays[1]

W. A. GERSDORFF and P. G. PIQUETT, *Entomology Research Division, Agric. Res. Serv., U.S.D.A.*

ABSTRACT

An analog of allethrin with the propargyl group replacing the allyl group as the side chain of the ketoalcoholic component was 64% as toxic as allethrin and 195% as toxic as pyrethrins to house flies (*Musca domestica* L.) by the turntable method. An analog with the phenyl group replacing the isobutenyl group of the acid component was 41% as toxic as allethrin and 120% as toxic as pyrethrins; however, it was only 34% as toxic as the corresponding isomeric form of allethrin.

Recently a sample of the *dl*-allethrolone ester of *dl-trans*-2,2-dimethyl-3-phenylcyclopropanecarboxylic acid, a synthetic pyrethroid prepared by Farkaš et al. (1958), was received from Dr. Jaroslav Weiser of Prague, Czechoslovakia, for testing against the house fly (*Musca domestica* L.) by the Campbell turntable method. Weiser (1958) found this compound highly effective against the same insect by other test methods—impregnated paper, Peet-Grady chamber, and topical application methods. However, the vehicle used in the Peet-Grady tests was ethyl alcohol, not refined kerosene as is commonly used in space sprays. The latter solvent was unavailable to him.

A pyrethroid, a propargyl analog of allethrin, prepared by M. S. Schechter and N. Green of this Division in unpublished work had indicated high toxicity to house flies in preliminary tests on the turntable.

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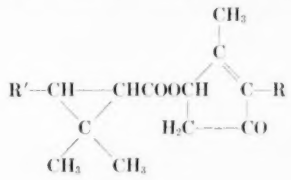
Table 1.—Toxicity relative to allethrin and pyrethrins of two new synthetic pyrethroids.

Material	Series	Regression Equations[a] Y	Standard Error of Slope	LC-50 (mg. per dl.)	Toxicity (%) Relative to— Allethrin	Pyrethrins
Propargyl analog	1	3.293X−1.660	0.119	105.3± 2.8	64.4±2.4	195.3± 6.8
Acid-phenyl analog	2	4.389X−5.501	.145	246.9± 5.8	40.8±1.4	119.7± 5.2
Allethrin	1	3.293X−1.032	.119	67.8± 1.9	100	303.1±10.8
	2	4.389X−3.790	.145	100.6± 2.4	100	293.7±13.0
Pyrethrins	1	2.390X−0.529	.082	205.5± 4.7	33.0±1.2	100
	2	3.291X−3.132	.203	295.5±10.9	34.0±1.5	100

[a] Y = mortality expressed in probits; X = concentration in mg. per deciliter, expressed as logarithms.

This paper reports bioassays made with these compounds in comparison with allethrin and pyrethrins.

The structural relationship of these compounds is shown below. The first compound, which for brevity may be referred to as an acid-phenyl analog of allethrin, must not be confused with an alcohol-phenyl analog (also shown) in which the substituting group is in position 2 of the ketoalcoholic component. The latter analog, prepared by Chen & Barthel (1953), has also been bioassayed by the turntable method (Gersdorff & Mitlin 1954).



Allethrin:
R is—$CH_2CH=CH_2$
R′ is—$CH=C(CH_3)_2$
Acid-phenyl analog:
R is—$CH_2CH=CH_2$
R′ is—C_6H_5
Alcohol-phenyl analog:
R is —C_6H_5
R′ is—$CH=C(CH_3)_2$
Propargyl analog:
R is—$CH_2{\equiv}CH$
R′ is—$CH=C(CH_3)_2$

The samples of the new esters and allethrin were nearly colorless, purified products. The same sample of allethrin was used in both series; it was of 93.4% purity as determined by the hydrogenolysis method. Two samples of pyrethrins were used; they were the unrefined extractives in which in the first series 60.5% and in the second series 54.2% of the total pyrethrins consisted of pyrethrin I and cinerin I as determined by the A.O.A.C. (Association of Official Agricultural Chemists) method.

The testing of the materials against house flies from a susceptible strain, necessarily done in two series, and the subsequent evaluation of relative toxicity, were made by the same methods described in previous work (Gersdorff & Piquett 1959).

Knockdown of flies was of high order for all materials; however, it fell to 30% with the lowest concentration of the acid-phenyl analog, 59 mg. per deciliter, whereas it was complete with the other materials at this concentration.

A summary of the results of the probit analysis of the mortality data is given in table 1.

DISCUSSION.—Another synthetic ester with a triple bond in the sidechain in position 2 of the ketoalcoholic component, the 2-butynyl analog of allethrin with the same synthetic acid component, was found by this method to be 73% as toxic as pyrethrins (Gersdorff & Mitlin 1951); however, with the propargyl (i.e., 2-propynyl) group in the same position the toxicity of the ester is about twice that of pyrethrins. Thus, the reduction from four to three carbon atoms in the side-chain with the triple bond, that bond becoming terminal, has resulted in a more toxic compound, just as it did in the similar case with the double bond, the change from cinerin I to the comparable *d-trans* acid form of allethrin (Gersdorff 1947, 1949).

When the phenyl group was substituted for the allyl group in position 2 of the ketoalcoholic component, the resulting ester was 34% as toxic as allethrin and 95% as toxic as pyrethrins by this method (Gersdorff & Mitlin 1954). In the present study when the phenyl group was substituted for the isobutenyl group in the acidic component, the resulting ester was 41% and 120% as toxic as the respective standards. However, the two phenyl analogs were not strictly comparable with respect to spatial configuration in the acidic components. The former was a mixture of the *dl-cis* and *dl-trans* forms, whereas the latter was the *dl-trans* form and would be more properly compared with *dl-trans*-allethrin rather than allethrin. Since *trans*-allethrin was found by this method to be 119% as toxic as allethrin (Gersdorff & Piquett 1958), the acid-phenyl analog was only 34% as toxic as the corresponding isomeric form of allethrin; therefore, it may be concluded that the effect on toxicity of the substitution of the phenyl group was the same in both cases.

Much importance has been ascribed to the presence of a double bond in the side chain in the acidic component. However, when the double bond in the isobutenyl group in allethrin was saturated, the resulting ester was but little less toxic than the acid-phenyl analog, 33% and 103% as toxic as the standards, allethrin and pyrethrins (LaForge et al. 1952). There is other evidence that, although of marked advantage, the presence of this double bond is not a prerequisite to high toxicity for such compounds (Gersdorff & Piquett 1959).

As estimated by this method, the propargyl analog is about as toxic as cyclethrin (Gersdorff & Piquett 1955) and the acid-phenyl analog about as toxic as furethrin (Gersdorff & Mitlin 1952).

The ratio of toxicity of the samples of allethrin and pyrethrins was in each series very close to the mean of all previous comparisons of these materials by this method (Gersdorff et al. 1961).

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A revision of this paper appeared in the BULLETIN OF THE ENTOMOLOGICAL SOCIETY OF AMERICA Volume 7, Number 2, pp. 59–62, June 1961. Authors and coauthors of manuscripts intended for publication by the Society should consult this revision carefully before preparing such manuscripts for publication

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SCIENTIFIC NOTES

Early-Season Collections of Three Cotton Insects by Argon-Glow Lamp and Black-Light Traps[1]

PERRY A. GLICK and HARRY M. GRAHAM, *Entomology Research Division, Agric. Res. Serv., U.S.D.A., Brownsville, Texas*

Light traps are useful in studying the distribution and abundance of several insect species. A trap which utilizes three 2-watt argon-glow lamps is being used extensively for surveying the distribution of pink bollworm (*Pectinophora gossypiella* (Saunders)). The trap is utilized in uninfested and eradication areas of the West (Berry et al. 1959) and also for following annual population trends at Brownsville, Texas. For these purposes traps that are effective with both high- and low-population densities are required.

Pfrimmer (1955) showed that a trap equipped with a 15-watt black light collected greater numbers of insects in all orders than one with three argon-glow lamps. However, of the black-light collections only 14% were Lepidoptera compared with 36% for the argon-glow lamp trap. For this reason the argon traps were used for pink bollworm collections. Although the numbers of Lepidoptera and other orders taken are less than those that would be taken by the black-light traps, the collections of other orders relative to those of Lepidoptera are decreased, thus facilitating examination of the collections. This factor is especially important during mid- and late summer when collections are very large. However, it is possible that during the spring, when the density of all populations is relatively low, the black-light trap could be used successfully to detect low populations of pink bollworm.

This study was initiated to determine the differences in numbers of adult pink bollworms collected by the black-light and argon-glow lamp traps during the early portion of the cotton-growing season (April 18 through June 30). Both types of traps (fig. 1) were omni-directional as described by Taylor et al. (1956). The main differences between the two were funnel size, and light radiation and wattage. The funnel diameters of the black-light and 3-lamp argon traps were 18 and 14 inches, respectively. According to Glick & Hollingsworth (1955), the black light used emitted a peak radiation of 3,500 angstroms in the ultraviolet range and a peak of 4,358 angstroms in the visible portion of the spectrum. The argon-glow lamps emitted a peak ultraviolet radiation of 3,654 angstroms and a visible peak of 7,500 angstroms. The total power tested was 15 watts for the black-light and 6 watts for the 3-lamp argon-glow traps.

The argon-glow and the black-light traps were placed 10 feet apart near a cotton field which had a relatively high early-season infestation of pink bollworm. The traps were operated on alternate nights through April, May, and June. The numbers of bollworm (*Heliothis zea* (Boddie)) and cabbage looper (*Trichoplusia ni* (Hübner)) were recorded, also.

The collections of pink bollworm by the black-light trap were

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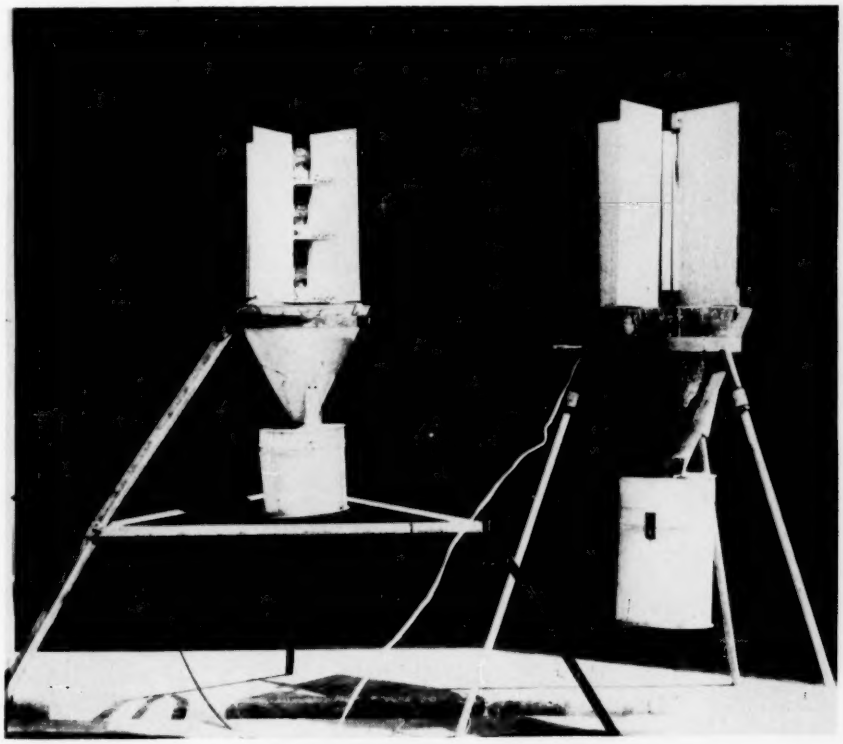

FIG. 1.—Three lamp argon-glow (left) and black-light (right) survey traps.

Table 1.—Early-season collections of three cotton insects by argon-glow lamp (A) and black-light (B) traps.

Month and Trap Type	Nights Operated	Mean Number of Insects per Night: Pink Bollworm Male	Pink Bollworm Female	Bollworm Male	Bollworm Female	Cabbage Looper
April						
A	6	0	0	0	0	1.17
B	7	0	0	0.57	0	1.14
Ratio A:B		0:0	0:0	0.57:0	0:0	1.0:1
May						
A	11	0.09	0	0.09	0	1.64
B	13	0.15	0	0.69	0	8.24
Ratio A:B		1.7:1	0:0	7.7:1	0:0	5.0:1
June						
A	15	1.33	2.40	1.07	2.60	17.53
B	15	4.20	6.00	14.53	15.33	172.00
Ratio A:B		3.2:1	2.5:1	13.6:1	5.9:1	9.8:1

larger than those by the argon-glow trap (table 1). The ratios of black-light collections to those by the argon-glow trap varied from 1.7:1 to 3.2:1. Collections of pink bollworm were extremely low by both traps in April and May. During this period development of the first and possibly a portion of the second generation occurred. Specimens were collected in both traps each month. Although the argon-glow trap caught fewer insects than the black-light, it may still be useful for survey purposes during the early season or with low population densities, especially when consideration is given to ease of examining the collections. It is possible that use of the black-light trap during the early season and the argon-glow later would be most advantageous when simple detection of species is wanted. However, this procedure would not be suitable when comparison of seasonal population trends is required.

Collections of bollworm and cabbage looper during the study are presented in table 1, also. The attractancy of the black light for these species relative to that of the argon-glow lamp was greater than that for pink bollworm.

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Studies on the Control of Thrips Attacking Onions[1]

James A. Harding[2]

The thrips (*Frankliniella occidentalis* (Pergande)) and the onion thrips (*Thrips tabaci* Lindeman) are the principal insect pests of economic importance attacking onions in the Winter Garden area of Texas. Research reports concerning the effect of thrips on onion yields are of a conflicting nature and apparently indicate a trend suggesting that as onion varieties and cultural methods were improved, thrips have had less effect on yields. The following citations form the basis for this indicated trend. Maughan (1933, 1934) showed increased onion yields from spraying onions due to a decreased thrips infestation. Hibbs & Ewart (1946) and Sleesman (1946) demonstrated this same phenomenon. Wilcox & Howland (1948) reported yield increases from thrips control, but not in every test as with earlier reports. In 1949, Douglass & Shirck had difficulties in producing yield differences with the more thrips-tolerant varieties as Sleesman (1943) had previously pointed out. During 1953, Richardson obtained significant differences between the treated plots and untreated plots, but failed to confirm these results in his 1954 studies. Shirk & Douglass (1956) reported their final studies on onion thrips in Idaho and of six tests on bulb yields, only two showed increases over the check plots. In nearly all of the aforementioned articles yields were correlated with thrips infestation levels, but reductions in yields depended on severe infestations and intermediate infestations resulted in no damage.

Table 1.—Effect of different thrips infestation levels on onion yields produced by weekly spraying of 1¼ pounds of malathion per acre for certain periods after transplanting during 1958–59 season.

Treatment: Weeks of Spraying	Date of Stopping	Mean No. of Thrips/10 Plants/Sampling Date: January 2	January 16	January 29	February 12	March 2	March 19	Total Thrips	Mean Yield[a] (lb./acre)
3	Jan. 8	1	16	11	41	201	137	407	38,042
6	Jan. 29	1	1	2	41	201	137	383	35,429
9	Feb. 19	1	1	2	21	201	137	363	39,422
12	Mar. 12	1	1	2	21	50	63	138	35,284
Untreated		12	16	11	41	201	137	418	37,825

[a] Ungraded onions.

Materials and Methods.—The tests were designed as randomized blocks in which treatments were replicated eight times in the two tests during the 1958–59 growing season, four times in the first test of the 1960–61 growing season, and three times in the 1960–61 test to compare effectiveness of insecticides. Plots in the 1958–59 tests were 20 feet long by four rows wide; 35 feet long by six rows wide in the 1960–61 yield test, and four rows wide by 35 feet long in the insecticide test. Guard rows were left between plots and all treatments were applied as sprays using one nozzle per row calibrated to deliver 5 gallons of spray mixture per acre.

Treatments were evaluated by recording the number of live thrips in the basal 6 inches of 10 consecutive plants per plot. The similarity in thrips numbers in tables 1 and 3 is due to a composite type of sample by counting the thrips in all plots treated the same and averaging the records for these plots. Yields reported herein are based on the ungraded onion bulbs from the

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[2] Assistant Entomologist, Texas Agricultural Experiment Station, Crystal City.

Table 2.—Effect of different thrips infestation levels on onion yields produced by spraying at different intervals, 1958–59 season.

Treatment[a] Interval	Mean No. of Thrips/10 Plants/Sampling Date: January 2	January 16	January 29	February 12	March 2	March 19	Total Thrips	Mean Yield[b]
7-day	2	1	3	10	22	60	98	30,129
14-day	2	17	9	15	33	100	176	31,363
21-day	2	15	12	28	52	114	223	30,274
Untreated	7	26	22	111	130	133	429	32,307

[a] Malathion, 1¼ pound per acre Dec. 18–March 12.

[b] Ungraded onions.

Table 3.—Effect of different thrips infestation levels on onion yields produced by various spray treatments during the 1960–61 season.

No. Treatments[a]	Mean No. of Thrips/10 Plants/Sampling Date									Total Thrips	Mean Yield[b] (lb./acre)
	March				April						
	20	24	27	31	3	7	10	14	17		
1	7	51	54	288	498	558	949	919	928	4,252	18,571
2	7	51	8	54	212	90	692	857	916	2,887	20,371
4	7	51	8	54	19	18	97	6	12	272	20,182
Untreated	285	685	711	953	877	646	1,002	977	973	7,109	16,866

[a] Parathion, 1¼ pound, + dieldrin, ¼ pound, at weekly intervals.
[b] Ungraded onions.

middle two rows of each plot. Data were analyzed statistically to determine any significant differences present.

The insecticides used in these tests included malathion, parathion, dieldrin, ethion, ronnel, Thiodan® (6,7,8,9,10-hexachloro-1,5,5*a*,6,9,9*a*-hexahydro-6,9-methano-2,4,3,-benzodioxathiepin-3-oxide), Guthion® (*O,O*-dimethyl *S*-4-oxo-1,2,3-benzotriazine-3-(4H)-ylmethyl phosphorodithioate), Perthane® (1,1-dichloro-2,2-bis(*p*-ethylphenyl) ethane), Dibrom® (1,2-dibromo-2,2-dichloroethyl dimethyl phosphate), Trithion® (*S*-(*p*-chlorophenylthio)methyl *O,O*-diethyl phosphorodithioate), Sevin® (1-napthyl *N*-methylcarbamate), Shell Development Compound 4402 (1,3,4,5,6,7,8,8 octachloro 3a,4,7,7a-tetrahydron-4,7-menthanophthalan), Bayer 29493 (*O,O*-dimethyl *O*-[4-(methylthio)-*m*-tolyl] phosphorothioate), and Zectran® (4-dimethylamino-3,5 xylyl methylcarbamate).

RESULTS AND DISCUSSION.—Records secured in a test designed to maintain some plots relatively insect free for specified periods after transplanting are presented in table 1. Analysis of the data indicated that significantly different thrips levels were established at the various ages of the transplants, but no differences appeared in yields.

Data secured in another test designed to investigate the intensity of an infestation on yields are shown in table 2. Highly significant differences in the number of thrips per plant were recorded, but no differences were noted between the yields caused by these trips infestation levels.

A summary of the records taken from plots having the heaviest thrips infestations occuring locally in the past 4 years is presented in table 3. Analysis of the data showed that different levels of thrips infestations resulted from chemical spray treatments, but yields were not significantly affected.

Table 4.—Effect of various toxicants on thrips infestations, 1960–61 season.

Insecticide	Active Ingredient per Acre (lb.)	Mean No. of Thrips/10 Plants (days after application)		
		1	4	7
Parathion+dieldrin	0.25+0.33	6.3	20.0	371.3
SD 4402	0.25	16.3	87.0	113.0
Dibrom	0.75	20.0	80.3	453.7
Trithion	1	21.0	95.7	235.7
Guthion	0.25	26.3	47.3	226.0
Ethion	0.5	26.7	265.0	619.0
Thiodan	0.5	31.3	74.3	294.7
Zectran	1	33.3	55.7	164.0
Ronnel	0.75	40.0	225.7	331.0
Perthane	1	64.3	117.7	454.7
Sevin	1	65.0	137.7	510.0
Bayer 29493	1	88.0	26.7	234.3
Check	—	264.3	392.3	802.3
L.S.D. at 5% level		26.9	55.4	64.0

Data from a chemical evaluation test are shown in table 4. The chemicals are arranged in order of effectiveness 1-day after treatment. Based on the analysis, SD 4402, Trithion, Guthion, Thiodan, Zectran, and Bayer 29493 show promise of being more effective in reducing a thrips infestation on onions and having the desired residual effectiveness than the currently recommended mixture of parathion and dieldrin.

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The Effect of Clean Culture and Early Spring Acaricide Treatments on Overwintering Spider Mite Populations in Outdoor Rose Plots[1]

T. J. HENNEBERRY and E. A. TAYLOR, *Entomology Research Division, Agric. Res. Serv., U.S.D.A.*

The two-spotted spider mite (*Tetranychus telarius* (L.)), a serious pest of outdoor roses, overwinters at Beltsville, Md., on annual winter weeds such as horseweed (*Erigeron canadensis* L.) and common chickweed (*Stellaria media* (L.) Cyrill). In early spring, the overwintered mites reinfest young foliage of rose plants and become established. This reinfestation extends the need for spider mite control to the entire growing season. Previous observations by investigators have indicated that removal of winter weeds from rose plots is a good supplemental measure to prevent early mite infestation (Taylor et al. 1956). The present

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study was conducted to determine the effectiveness of clean cultural practice (removal of winter weeds in the fall) and the application of acaricides, in the spring, before the first foliage appears, for the protection of rose plants from reinfestation by overwintered mite populations.

The experimental planting was arranged in a split-plot design in four replications as described by Cochran & Cox (1959). Whole plots contained eight subplots, and each subplot consisted of six rose plants, three each of the varieties Red Radiance and Helen Traubel.

In October, 1957, half of the whole plots was kept free of weeds throughout the fall and the following spring months. The remaining whole plots were left unweeded; in addition, common vetch (*Vichia sativa* L.) was planted to provide more live plant material for overwintering mite populations.

The acaricide treatments consisted of a 2×2 factorial arrangement of a wettable powder containing 15% of Aramite® (2-(*p-tert*-butylphenoxy)-1-methylethyl 2-chloroethyl sulfite) and a wettable powder containing 25% of Tedion® (2,4,4′,5-tetrachlorodiphenyl sulfone) applied to subplots of each half of the whole plots. Both acaricides were applied at the rate of 2 pounds of wettable powder per 100 gallons of water with 2-gallon compressed-air sprayers at 60 p.s.i. On April 28 dormant canes were thoroughly wetted, and the ground and weeds around the plant were also covered although no attempt was made to actually direct spray on these areas.

Spider mite population counts were made on two sampling dates, May 26 and June 9. The total number of living mite forms was counted on each of five leaf discs taken at random from plants in each of the subplots.

On May 26, rose plants in the weed-free plots had only one-third as many spider mites as those in plots with weeds (fig. 1). Rose plants in plots with weeds and not treated with Tedion sprays showed extreme mite-feeding injury that caused the mite populations to decline. Therefore, the effect of clean culture was not apparent after the May 26 sampling. On June 9, the mite populations declined on the Tedion and the Aramite-Tedion-treated plants. This decline resulted from eggs that failed to hatch or a high mortality of the hatching larvae.

Sprays of Tedion or Aramite-Tedion on dormant canes, with or without clean culture, reduced mite populations through the first week in June. These spray treatments plus clean culture were highly effective.

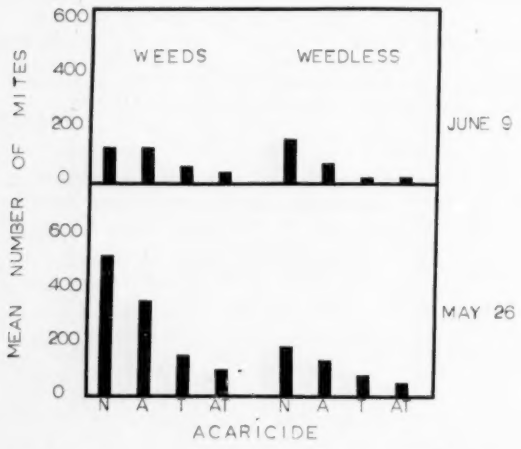

FIG. 1.—Mean number of mites per plot after clean culture alone or in combination with various early spring acaricide treatments: N, None; A, with Aramite; T, with Tedion; AT, with Aramite-Tedion.

The results of the present study support the practice of clean culture to prevent the establishment of spider mites in rose plantings early in the growing season. This practice, combined with an early spring application of Tedion or Tedion plus Aramite to dormant canes, provided protection from mite infestation, and made the problem of control during the growing season easier to accomplish. Early season defoliation of rose plants from blackspot has been reported to effect winter hardiness more than late season defoliation (Carrier 1953). The benefits of early season protection to prevent the buildup of mite populations and reduce plant injury is particularly desirable, since injury caused by spider mites has also been found to reduce plant vigor and affect winter survival of rose canes (McClellan et al. 1957, Henneberry et al. 1961).

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A Method for Rearing the Lettuce Root Aphid, *Pemphigus bursarius*[1]

D. L. McLEAN and M. G. KINSEY,[2] *University of California, Davis*

A chamber was designed to rear the lettuce root aphid, *Pemphigus bursarius* (L.), in large numbers for use in studying the aphid as a vector of lettuce mosaic virus. In addition to rearing,

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[2] Assistant Entomologist and Laboratory Technician, respectively, Department of Entomology and Parasitology, University of California, Davis.

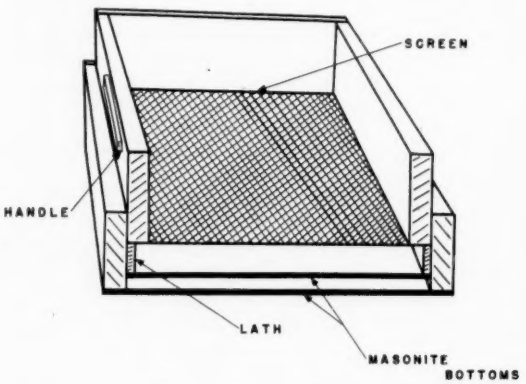

FIG. 1.—A diagramatic sketch of the root aphid rearing chamber. The proximal end has been removed to show the construction. Measurements are given in the text.

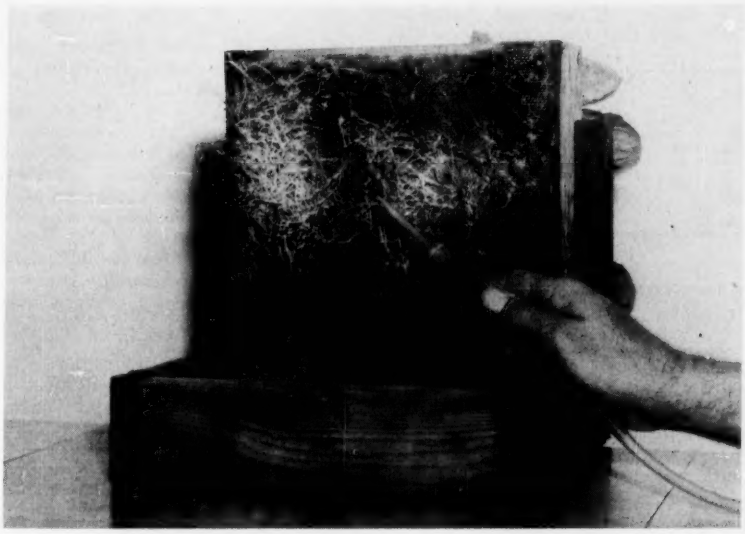

FIG. 2.—The top frame of the chamber standing on end to show the root aphid colony and the method used for aspiration of the aphids.

the chamber was also designed to facilitate collection of the aphids.

PROCEDURE.—The chamber was constructed in two parts so that one frame was within another frame (fig. 1). The top frame was 9½ inches square made from 3½- by ¾-inch redwood lumber. The bottom frame was 11 inches square constructed also of redwood. Galvanized or aluminum nails were used for frame assembly. Aluminum screen of the type and gauge used commonly as window screening was attached with aluminum tacks to the bottom of the top frame.

Redwood lath, ¼ by 1 inch, was nailed 1½ inches from the top to two inner and opposite walls of the bottom frame to act as a support for the top frame. Because the top frame contacted only the two pieces of lath, there were small areas between the top frame and the bottom frame that allowed for escape of the alates to the outside. This was desired since alates were also used in the vector study. If complete confinement of emerging alates is desired, redwood lath can be placed on all four inner walls. Plastic self-adhering weather stripping can be applied to the upper surface of the lath to seal the aphids in the lower frame. A bottom of tempered masonite was nailed to the lower surface of the lath.

Soil was placed in the top frame and lettuce seeds were planted in the soil about one-half inch apart. The aphids reproduced faster when Great Lakes variety lettuce was used. A double layer of paper toweling was placed on the masonite bottom in order to retain moisture.

After a number of roots had grown through the screen and were long enough to reach the toweling, aphids were introduced onto the toweling. The chamber was watered from the top with no apparent ill effect on the aphids.

When aphids were desired for testing, the top frame was removed, placed on end and the desired number of aphids easily aspirated from the colony (fig. 2).

This chamber, or modifications of it, could be successfully used for rearing other root feeding insects or arthropods.

Development of Insecticide Resistance in Three Field Strains of House Flies[1]

G. C. LABRECQUE, and H. G. WILSON, *Entomology Research Division, Agric. Res. Serv., U.S.D.A.*

Since 1951, poison baits have been the preferred means for control of house flies (*Musca domestica* L.) in dairy barns of Central Florida. Malathion and Dipterex® (*O,O*-dimethyl 2,2,2-trichloro-1-hydroxyethyl phosphonate) are the principal insecticides used in these baits. Where flies have developed resistance to malathion, DDVP has been used with malathion or has replaced it.

Laboratory tests have indicated that house fly resistance to malathion can be induced readily. Susceptible flies subjected to malathion pressure in the adult stage developed 2-fold resistance to this insecticide within 17 generations and 100-fold resistance by the 60th generation (LaBrecque & Wilson 1960). Flies that showed 10-fold resistance to Dipterex when collected in the field and that were continually exposed to Dipterex mixed with the adult food showed no additional increase in resistance for 98 generations (Wilson et al. 1959).

In 1954, and annually from 1956 through 1960, tests were made with flies collected in each of three dairy barns near Orlando, Florida, to follow the development of resistance to malathion and Dipterex in the field. Each summer flies were collected in the barns, temporary colonies were established, and resistance tests were made with flies of the F_1 or F_2 generations. The tests included exposures to baits and usually to contact sprays. A few tests were also made with residues of Dipterex.

In the bait tests acetone solutions containing various concentrations of malathion or Dipterex were added to dry sugar and the mixtures were allowed to dry. Ten-gram samples were spread over the bottoms of petri dishes, cylindrical screen cages containing 20 female flies were placed on the baits so that the insects could feed ad libitum through the screen, and the mortality was determined after 24 hours. Cotton saturated with water was placed on the tops of the cages after 4 hours. LC-50's and LC-90's were computed from the concentration-mortality data. The ratio

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Table 1.—Ratios of resistance of three field strains of house flies to malathion and Dipterex baits and contact sprays in various years.[a]

STRAIN	1954	1956	1957	1958	1959	1960
Dipterex Bait						
Hiatt	6 (8)	3 (5)	5 (>22)	11 (6)	3 (5)	11 (19)
Arndt	6 (8)	4 (8)	6 (22)	16 (6)	6 (10)	18 (36)
Lakemont	7 (9)	3 (9)	3 (8)	17 (6)	4 (4)	> 50 —
Malathion Bait						
Hiatt	— —	4 (4)	19 (>45)	56 (>56)	31 (607)	>275 —
Arndt	— —	4 (7)	30 (>45)	>40 —	163 (943)	>275 —
Lakemont	— —	4 (>4)	13 (19)	>40 —	27 (100)	>275 —
Dipterex Contact Spray						
Hiatt	— —	— —	29 (>29)	12 (14)	9 (45)	11 (31)
Arndt	— —	— —	3 (>5)	8 (8)	12 (15)	8 (22)
Lakemont	— —	— —	12 (>12)	8 (9)	16 (24)	9 (28)
Malathion Contact Spray						
Hiatt	— —	— —	3 (>3)	6 (12)	14 (12)	23 (>23)
Arndt	— —	— —	3 (>5)	12 (37)	14 (30)	19 (>20)
Lakemont	— —	— —	7 (>7)	21 (>21)	15 (19)	11 (>20)

[a] Ratios of the LC-50 obtained with the field strain to that obtained with the normal laboratory strain and, in parentheses, ratio of the LC-90's.

of the LC-50 or LC-90 to that obtained in concurrent tests with flies from the normal colony indicated the degree of resistance.

Contact-spray tests were run in the wind tunnel. Female flies, 4 or 5 days old, were exposed to various concentrations of the insecticide in odorless kerosene solutions. One-fourth ml. of solution was atomized at a pressure of 1 p.s.i. into the mouth of the tunnel and drawn by a 4-m.p.h. draft through a screen-wire cage containing the flies. After treatment the flies were transferred to screen holding cages and supplied with a 10% honey solution on absorbent pads. The mortality was recorded after 24 hours. Duplicate tests were run at each concentration. LC-50's and LC-90's were computed and the degree of resistance was determined in the same manner as in the bait tests.

The results with baits and contact sprays are summarized in table 1. Usually, but not invariably, the resistance ratio was higher at the LC-90 level than at the LC-50. In some series of tests 90% mortality was not obtained and the resistance at the LC-90 level could not be expressed accurately as a ratio. For this reason the following discussion of resistance trends is based on the ratios at the LC-50 level only.

Flies exposed to Dipterex baits in 1954 showed 6- to 7-fold resistance. Resistance to Dipterex fluctuated from 3- to 17-fold between 1956 and 1959 but reached more than 50-fold in one strain in 1960. Resistance to malathion baits increased more or less steadily from 4-fold in 1956 to more than 275-fold by 1960.

Contact-spray evaluations were initiated in 1957. Resistance to Dipterex ranged from 3- to 29-fold in 1957 and fluctuated between 8- and 16-fold thereafter. Resistance to malathion ranged from 3- to 7-fold in 1957 and increased progressively, except in the Lakemont strain, to 11- to 23-fold by 1960.

In the residue tests acetone solutions of Dipterex were sprayed on plywood panels at the rate of 100 mg. of active ingredient per square foot. Exposures were made by confining 20 female flies, 4 or 5 days old, under petri-dish halves on the treated surfaces for various periods of time. The flies were then transferred to clean holding cages, given food and water, and held for the 24-hour mortality counts. Duplicate groups of flies were tested at each exposure. The time-mortality data were inadequate for the computation of LT-50's, and the estimation of resistance was based on the exposure necessary to obtain 65% to 100% 24-hour mortality of flies from the field strains and the normal laboratory colony. Resistance in the field strains was less than 2-fold in 1954 and varied between 2- and 8-fold from 1957 to 1960.

The high resistance exhibited to malathion in baits may be attributable in part to behavioral as well as physiological resistance to this insecticide (Kilpatrick & Schoof 1958), although behavior was not an important component in the degree of resistance shown by another strain collected near Orlando (Schmidt & LaBrecque 1959).

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Larvae of *Nomophila noctuella* Feeding on Douglas-fir Nursery Seedlings[1]

NORMAN E. JOHNSON[2] and JOHN W. DUFFIELD[3]

Damage to seedlings of Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco.) by the larvae of *Nomophila noctuella* (Denis & Schiffermüller) (Lepidoptera: Pyraustidae) in the Industrial Forestry Association's Col. William B. Greeley Forest Nursery near Olympia, Washington, was first reported in the summer of 1958 (U. S. Dept. Agric. 1958, Weyerhaeuser Timber Co. 1959). A number of larvae were collected and reared to adults which were identified to species by H. W. Capps of the U. S. Department of Agriculture.

In the nursery, damage was confined to 1-year-old seedlings which were clipped off neatly ¼ to ¾ inch above the ground leaving a bare stalk. Some of the needles may be consumed at the point of clipping, but most of them are dragged into the larval tunnels. These tunnels are constructed in the soil 1 to 2 inches deep and are often lined with Douglas-fir needles interwoven with silk spun by the larva (fig. 1). The larva proceeds outward from the tunnel leaving a strand of silk along its path. As many as 15 seedlings were observed to have been cut by a single larva and damage estimates approached 5% in some of the 1,000-square-foot nursery beds. This damage is very similar to Eidt's (1960) report of this unusual nursery pest's destruction of red and white spruce seedlings in New Brunswick. Unless the soil tunnels are found, one might mistake the damage for that caused by cutworms. The cutworms consume the foliage of the seedling without dragging it into a tunnel and do not leave the characteristic silk strands.

This insect is a rather general feeder, having been reported on a number of agricultural crops and wild herbs. Eidt (1960) induced the insect to feed on eastern white cedar, balsam fir, yellow birch, choke cherry, and mountain ash.

Biology of the insect has been studied by Felt (1893), Flint (1922), and Smith (1942). *Nomophila noctuella* is widely distributed throughout the world. There are from two to four generations per year. Eggs are laid on stems and foliage of plants where they hatch in about 10 days. The larvae apparently have five instars, and, on maturity, pupate in the soil nests or occasionally in the axils of leaves of infested plants. Under optimum conditions only 10 days are required for the pupal period. The insects overwinter as larvae in the soil and litter. Eidt (1960) reports that the insect is migratory and populations characteristically fluctuate widely.

The adult moth is ½ to ¾ inch long with a wing spread up to 1¼ inches. The fore wings are gray with black and bronze markings. Comstock (1925) gives a diagram of the wing venation of this

[1] Accepted for publication July 17, 1961.

[2] Forest Entomologist, Weyerhaeuser Forestry Research Center, Centralia, Washington.

[3] Technical Director, Col. William B. Greeley Forest Nursery, Nisqually, Washington.

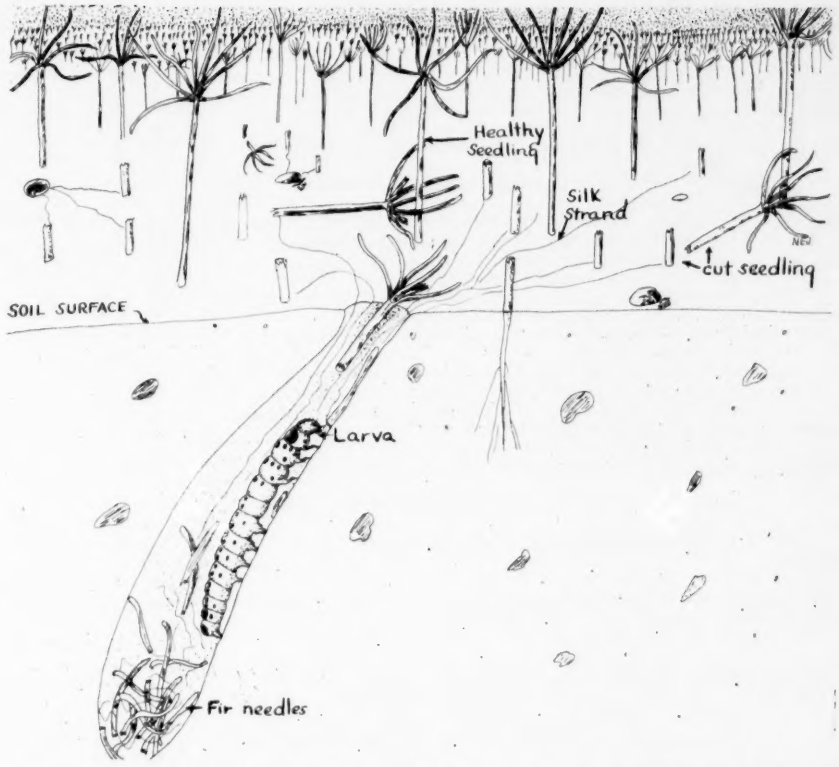

FIG. 1.—Schematic drawing of larva of *Nomophila noctuella* in soil tunnel showing damaged and healthy Douglas-fir seedlings in background.

species (fig. 790, p. 645). The moths are also characterized by their pattern of flying. When disturbed they fly quickly to a spot 6 to 10 feet away and alight much as a grasshopper. The mature larva is ½ to ⅞ inch long, gray green with numerous dark spots on each body segment and a black thoracic shield and head capsule. When disturbed, larvae move quickly with a characteristic vigorous backward motion. Ellis (1925) gives a more complete description of the eggs and larvae.

The larvae crawl around on the surface of the soil, where chemical control should be effective. In the Greeley Nursery, damage ceased after application of heptachlor. The formulation used was an emulsifiable concentrate containing 2 pounds of heptachlor per gallon of concentrate. Two pints of concentrate were mixed in 400 gallons of water, and applied at the rate of 10 gallons per 1,000 square feet of nursery bed, using tractor-drawn spray equipment.

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The Toxicity of Some Phosphorothioic Acid Esters to the Two-Spotted Spider Mite[1]

B. A. BUTT and J. C. KELLER, *Entomology Research Division, Agric. Res. Serv., U.S.D.A.*

From 1956 to the present time a number of phosphorothioic acid esters have been submitted from industrial sources for preliminary laboratory testing at the Brownsville, Texas, laboratory of the Entomology Research Division. This paper reports the results of these tests against two-spotted spider mites (*Tetranychus telarius* (L.)).

The mites used in these tests were reared on cotton seedlings in a constant-temperature chamber (82° F. and 70% to 100% relative humidity). Mite-infested seedlings were placed on a turntable in a horizontal wind tunnel (air speed 3.4 m.p.h.) and sprayed with 5 ml. of an acetone solution of the acaricide. After treatment the seedlings were removed from the wind tunnel and held in the laboratory at 80° F. Mortality of mites was recorded

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Table 1.—Results of tests with some phosphorothioic acid esters against two-spotted spider mites.

Insecticide	Chemical Name	Per Cent Kill in 48 Hours at Indicated Per Cent Concentration					
		1	0.5	0.25	0.1	0.05	0.01
Cyanamid 12503	*O,O*-diethyl *O*-[2 phenyl-6-(3(2*H*)-pyridazinonyl)] phosphorothioate	98	96	93	98	100	100
Chipman 6200[a]	*O,O*-diethyl *S*-(2-diethylamino)ethyl phosphorothioate, *p*-toluenesulfonate	100	97	96	95	99	98
Stauffer R-1505	*O,O*-diethyl *S*-phthalimidomethyl phosphorothioate	100	100	99	98	96	97
Bayer 25198	*O,O*-dimethyl *O*-(*p*-methylsulfinyl)phenyl phosphorothioate	100	100	98	98	96	81
Stauffer R-1571	*O,O*-dimethyl *S*-phthalimidomethyl phosphorothioate	100	100	100	100	100	75
Geigy-24027	*O,O*-diethyl *O*-(3-methyl-5-pyrazolyl) phosphorothioate	100	87	97	100	98	43
Bayer 29709	*O*-(*p*-methylsulfinylphenyl) *O,O*-dipropyl phosphorothioate	99	86	81	39	6	—
Monsanto CP-11549	*O,O*-diethyl *S*-(2-propynyl) phosphorothioate	98	81	—	—	—	—
Union Carbide C&C 8305	*P*-chloro-5-methyl-*P*-thio-2,4,3-dioxaphosphabicyclo[4.4.0] decane	100	68	60	—	—	—
Bayer 25142	*O,O*-diisopropyl *O*-(*p*-methylsulfinyl)phenyl phosphorothioate	99	67	—	—	—	—
Bayer 29493	*O,O*-dimethyl-*O*-(4-methylthio-*m*-tolyl) phosphorothioate	97	63	64	—	—	—
Bayer 25141	*O,O*-diethyl *O*-(*p*-methylsulfinyl)phenyl phosphorothioate	93	60	—	—	—	—
Monsanto CP-11903	*O,O*-diethyl *S*-pentachlorophenyl phosphorothioate	91	61	—	—	—	—
Bayer 22893	*O,O*-diethyl *O*-pinacolyl phosphorothioate	82	—	—	—	—	—
Atomic Basic Chemicals X-500	*O,O*-diethyl *O*-(*m*-pentadecylphenyl) phosphorothioate	74	—	—	—	—	—
Dition	*O,O*-diethyl *O*-(3,4-tetramethyleneumbelliferone) phosphorothioate	65	—	—	—	—	—
Bayer 22408	*O,O*-diethyl *O*-naphthalimido phosphorothioate	32	—	—	—	—	—
Malathion standard		—	95	82	59	42	—
Schradan standard		—	—	71	32	—	—

[a] Compound no longer available.

after 48 hours by microscopic examination of the cotton seedlings. Materials that failed to kill 90% of the mites at a concentration of 1% were not tested further. Malathion and schradan were standards of comparison and the tabular data on these standards are averages of a large number of tests.

The results of these tests are summarized in table 1.

American Cyanamid 12503, Chipman 6200, Stauffer R-1505, Bayer 25198, and Stauffer R-1571 were the most effective acaricides. With these compounds 75% to 100% of the mites were killed at the lowest test dosage of 0.01%. Geigy 24027 was only slightly less effective; it produced a 43% kill at 0.01%. All of these compounds were more effective than the standards. Bayer 29709 was about equal to the malathion standard at a concentration of 0.25%. CP-11549 and Union Carbide C&C 8305 were less effective than the malathion standard. Bayer 29493, Bayer 25142, Bayer 25141, Monsanto CP-11903, Bayer 22893, Atomic Basic Chemicals X-500, dition, and Bayer 22408 were less effective than the schradan standard.

Use of P^{32} as an Aid in Biological Studies of the Leafhopper, *Scaphoideus luteolus*[1]

C. JOHN HAY[2] and WILLARD C. MYSER[3]

Radioactive tracers are now commonly used in studying the life histories of insects. Many insects have been successfully tagged with P^{32} (PO_4 in weak HCl). Recently we investigated the possibility of using this isotope to tag the insect vector *Scaphoideus luteolus* Van Duzee of elm phloem necrosis and to trace this leafhopper's oviposition habits through activated eggs.

PROCEDURE.—Elm (*Ulmus* spp.) plants 1½ to 2 feet high with at least two stems ⅛ to ¼ inch thick were selected from a stock of 500 virus-free potted plants. Each potted plant was placed in a tile dish lined with aluminum foil and its lower foliage pruned. The latter was done to force a concentration of plant activity in to the uppermost foliage. Continuous exposure of the activated plants to overhead fluorescent light, with no side light, helped to bring about this concentration more rapidly.

Each plant was activated in the following manner: One stem was cut just below a node near the junction with the other stem. The end of the cut stem was joined to the large end of a 3-inch medicine dropper, rubber bulb removed, by means of a piece of latex rubber tubing ¾ inch long (⅛-inch opening), thus making a leak-proof connection. The radioactive solution was dropped into the small end of the dropper with a hypodermic syringe. Each plant received a 1- or 2-cc. dosage containing, respectively, 1 or 2 millicuries of P^{32}.

As soon as all the radioactive solution was absorbed, the plants were placed in portable screened cages, 10 by 10 by 30 inches. Late instar nymphs were released in the cages and allowed to feed until they molted into adults. After hardening up, the adults were removed from the activated plants. All the males were destroyed. Radiation of the females was measured with the probe of a portable beta-gamma survey meter held about three-eighths of an inch from the insect. Those registering 5,000 counts per minute or more were used in the tests; the rest were returned to the activated plants.

Two lines of study were used to follow the egg-laying habits. In one, radioactive females were released with nonradioactive males in 10- by 10- by 30-inch plastic screened cages each of which contained a potted, nonactivated elm plant along with one or more elm bolts (3 by 24 inches). In the other, activated females were released with nonactivated males in a walkin, plastic-screened cage, 18 by 24 by 7 feet. This cage housed planted elms and bolts of elm, hackberry (*Celtis occidentalis* L.), black willow (*Salix nigra* Marsh.), osage-orange (*Maclura pomifera* (Raf.) Schneid.), and white ash (*Fraxinus americana* L.). The last mentioned four species were included to find out if the leafhopper would lay eggs in bark of species other than elm. According to Baker (1949), the eggs of *S. luteolus* are found buried in the cork parenchyma of elm bark. These were therefore chosen because of their roughened,

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[2] Entomologist, U. S. Dept. of Agriculture, Forest Service, Central States Forest Experiment Station, Forest Insect Research Laboratory, Delaware, Ohio.

[3] Associate Professor of Zoology and Entomology, Ohio State University, Columbus.

corklike, furrowed bark and because they grow naturally in association with elm.

RESULTS.—Some of the plants that were treated with 2 millicuries of radioactive P^{32} absorbed the solution in 2 to 3 hours. Leafhoppers feeding on these plants reached a radioactive level of more than 30,000 c.p.m. per individual in a few days and reached the maximum found (80,000 c.p.m.) in about 8 days. On plants that took 50 to 100 hours to completely absorb the solution, it took the insects about 11 days of feeding to reach a fairly high (5,000 to 10,000 c.p.m.) radioactivity level.

No ill effects to the activated leafhoppers could be detected. Mortality was comparable to that of the nonactivated checks. Death resulted naturally or because of injury during handling. The females attained an activity approximately twice that of the males, probably because of their larger size. By dissecting 64 females that had acquired their radioactivity in the nymphal stage we found that the average activity per egg was 1/36 that of the female body and ranged from 1/24 to 1/50. Few of the eggs studied exceeded 100 c.p.m.; most were around 50 c.p.m.

Only the elm bolts in the small cages contained radioactive eggs. Eggs were laid 8 to 30 days after mating and could be traced with the probe of the survey meter to within one-half inch of their exact location. The eggs were laid singly in the corky layer of the bark with one or more eggs per spot. Only 18% of the radioactive spots contained eggs, however; the rest of the spots were contaminated by honeydew. It is probable that eggs were laid after the 30-day period, but no more spots containing eggs could be detected, even though eggs were still present in the females. Failure to detect eggs after 30 days is attributable to the short half life of the isotope (14.7 days) and to the fact that much activity had been eliminated in the honeydew.

Counts were not made in the walkin cage until after 30 days. At this time we were unable to discover any eggs. This may be partially explained by the fact that the isotope had decayed through two or three half lives and much radioactivity had been eliminated by honeydew excretions.

More activity probably could have been attained in the eggs if the females had been kept on the activated elm plants for 2 to 4 weeks past the nymphal stage. After mating, it appears to take several weeks for the eggs to develop and for egg laying to begin.

The use of P^{32} as an aid in biological studies of *S. luteolus* shows promise. The leafhoppers can be labeled easily with sufficient radioactivity to trace oviposition habits. However, further refinement of the technique would be necessary before results would be completely satisfactory.

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Laboratory Tests with Toxaphene, DDT, and Sevin against *Lygus hesperus* on Cotton[1]

G. T. BOTTGER, and A. N. SPARKS, *Entomology Research Division, Agric. Res. Ser., U.S.D.A., Tucson, Arizona*[2]

In 1960 and 1961 toxaphene, DDT, and Sevin® (1-naphthyl *N*-methylcarbamate), and mixtures of toxaphene-DDT and toxaphene-Sevin in a 3–1 ratio of the technical materials, were tested in the laboratory at Tucson to determine their comparative effectiveness against *Lygus hesperus* Knight, an important insect pest of cotton in Arizona.

Technical DDT and toxaphene were dissolved in xylene to make stock solutions. With Sevin, the 85% sprayable formulation was used. Sprays were prepared by mixing a sufficient amount of stock solution or concentrate with distilled water, containing 1.5% of the emulsifier Triton X-151, to give the desired strengths. The sprays were applied to greenhouse-grown potted cotton plants with a DeVilbiss hand-atomizer nozzle, at 20 p.s.i. Residues were uniformly distributed on both sides of the leaves. Immediately after the plants were sprayed they were moved to a holding room at a temperature of 80±2° F. and relative humidity ranging from 20% to 50%. Fifteen to twenty 2-day-old adult lygus bugs reared in the laboratory were caged with each plant. From 12 to 15 plants were used in each test. Mortalities recorded 72 hours after treatment were corrected by Abbott's formula (Abbott 1925).

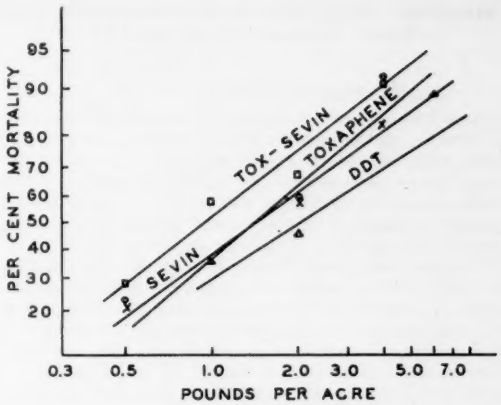

FIG. 1.—Dosage-mortality curves for adult lygus bugs exposed 72 hours to cotton plants sprayed with DDT, Sevin, toxaphene and a 3:1 mixture of toxaphene and Sevin.

The average per cent mortality obtained at each dosage level was plotted on log-probit paper, and the dosage-mortality regression curves were fitted by eye (fig. 1).

The approximate LD-50's in pounds per acre were DDT 2.0, Sevin 1.6, toxaphene 1.4, toxaphene-DDT 1.5, and toxaphene-Sevin 1.0. Because the toxaphene-DDT regression line was so similar to that of toxaphene, it was omitted from the figure for reasons of legibility. Average per cent mortalities for 0.5, 1, 2, and 4 pounds of toxaphene-DDT were 16, 38, 57, and 84, respectively.

The most effective treatment, the toxaphene-Sevin mixture, warrants field testing. Since the mixture was more effective than either of its components, synergism was indicated. This mixture should be highly effective against a complex of some of the major cotton insect pests in Arizona. Bottger et al. (1958) showed in laboratory and field tests that Sevin was effective against the bollworm (*Heliothis zea* (Boddie)), and the pink bollworm (*Pectinophora gossypiella* (Saunders)). They also showed in laboratory tests that this insecticide was effective against the salt-marsh caterpillar (*Estigmene acrea* (Drury)) and the cotton leafworm (*Alabama argillacea* (Hübner)). Wene et al. (1960) showed that it was highly effective against the salt-marsh caterpillar in field tests in Arizona.

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[2] In cooperation with the Arizona Agricultural Experiment Station.

Laboratory Tests with Some Phosphoric Acid Esters against Cotton Aphids[1]

J. C. KELLER and B. A. BUTT, *Entomology Research Division, Agric. Res. Serv., U.S.D.A.*

From 1956 to date entomologists at the Brownsville, Texas laboratory of the Entomology Research Division, have had the responsibility of conducting preliminary laboratory tests against several cotton insects with new insecticides submitted from various sources for evaluation. The purpose of this paper is to report the results of tests with some phosphoric acid esters against the cotton aphid (*Aphis gossypii* Glover).

The aphids used in these tests were reared on cotton seedlings in a constant-temperature cabinet at 67° F. without humidity control. In carrying out the tests, seedlings infested with 20 or more cotton aphids were placed on a turntable (24 r.p.m.) in a horizontal wind tunnel (air velocity 3.4 m.p.h.) and sprayed with 5 ml. of acetone solution of the insecticides. After treatment the seedlings were removed from the wind tunnel and held in the laboratory at 80° F. Mortality was recorded after 24 hours. Schradan and malathion were used as standards. Compounds that caused less than 90% mortality at a 1% concentration were not tested further.

The results of the tests are summarized in table 1.

Shell SD-4092, SD-4239, and General Chemical 3661 were the most effective sprays that produced complete kill of aphids at the lowest test dosage of 0.01%. Shell BAS-4294, BAS-4475, and SD-2244 were 96% to 97% effective at the lowest test dosage. Shell SD-3562, SD-5539, Hercules 3895, and Monsanto CP-10502 were 80% to 88% effective at the lowest test dosage. All of the compounds were more effective than the schradan and malathion standards. Hercules 3004 was 84% effective at 0.015%. General Chemical 3582, Shell SD-5533, General Chemical 3583, phosphamidon, General Chemical 4072, Monsanto CP-10516 and CP-12432 produced kills of 75% to 97% at a concentration of 0.25%. Bayer 22684, 24869, Dibrom, Heyden Newport Newphos No. 1, General Chemical 3707, and Bayer 25820 produced kills ranging from 54% to 92% at a concentration of 1.0% and were the least effective of the compounds tested.

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The Effect of Centrifugal Force on Pink Bollworm Larvae[1]

EDGAR W. CLARK[2] and CLYDE A. RICHMOND, *Entomology Research Division, Agric. Res. Serv., U.S.D.A., Brownsville, Texas*[3]

The lethal effect of centrifugal forces on insects has had little study. Sullivan & Westlake (1959) raised two generations of vinegar flies (*Drosophila melanogaster* Meigen) under a continual stress of 10 g's (earth's gravity) with no apparent ill effects other than an increased developmental time from egg to adult and a reduced yield of insects. Sullivan & McCauley (1960) found that the median lethal relative centrifugal force for confused flour beetles (*Tribolium confusum* Jacquelin du Val) was 20,600 g's for 73 minutes, house flies (*Musca domestica* L.) 11,400 g's for 2 minutes

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[2] Now with Forest Service, Southeastern Forest Experiment Station, Asheville, N. C.
[3] In cooperation with the Texas Agricultural Experiment Station.

Table 1.—Per cent mortality of cotton aphids 24 hours after treatment in the laboratory with several phosphoric acid esters dissolved in 5 ml. of acetone (single tests with 20 or more aphids).

Insecticide	Chemical Name	Per Cent Kill at Indicated Concentration					
		1.0	0.5	0.25	0.1	0.05	0.01
Shell SD-4092	1-benzyloxycarbonyl-1-propen-2-yl dimethyl phosphate	100	100	100	100	100	100
SD-4329	1-(*p*-chlorobenzyloxycarbonyl)-1-propen-2-yl dimethyl phosphate	100	100	100	100	100	100
Gen. Chem. 3661	1,3-bis(methoxycarbonyl)-1-propen-2-yl diethyl phosphate	100	100	100	100	100	100
Shell BAS-4294	dimethyl 1-(*alpha*-methylbenzyloxycarbonyl)-1-propen-2-yl phosphate	100	96	98	99	99	97
BAS-4475	1-(2,4-dichlorobenzyloxycarbonyl)-1-propen-2-yl dimethyl phosphate	98	99	89	97	98	97
SD-2244	diethyl 1-(dimethylcarbamoyl)-1-propen-2-yl phosphate	100	91	93	94	—	96
SD-3562	dimethyl 1-(dimethylcarbamoyl)-1-propen-2-yl phosphate	100	100	97	99	—	88
SD-5539	dimethyl 1-(*m*-nitrobenzyloxycarbonyl)-1-propen-2-yl phosphate	100	100	100	98	94	82
Hercules 3895	2,2-bis(ethylthio)vinyl diethyl phosphate	100	100	100	91	—	87
Monsanto CP-10502	1-(dimethoxyphosphinyl)vinyl dimethyl phosphate	100	100	99	98	100	80
Hercules 3004	diethyl 1-ethylthio-1-propen-2-yl phosphate	100	100	100	100	100	—
Gen. Chem. 3582	1-(2,5-dichlorophenyl)-2,2dichlorovinyl diethyl phosphate	100	100	97	90	53	16
Shell SD-5533	dimethyl(1-*p*-nitrobenzyloxycarbonyl)-1-propen-2-yl phosphate	100	94	95	15	—	—
Gen. Chem. 3583	2-chloro-1-(2,5-dichlorophenyl)vinyl diethyl phosphate	100	97	93	—	—	—
Phosphamidon		98	99	99	93	89	—
Monsanto CP-10516	1-(diethoxyphosphinyl)vinyl dimethyl phosphate	100	94	88	—	—	—
Gen. Chem. 4072	2-chloro-1-(2,4-dichlorophenyl)vinyl diethyl phosphate	100	97	87	—	—	—
Monsanto CP-12432	1-(dimethoxyphosphinyl)vinyl diethyl phosphate	100	85	75	—	—	—
Bayer 22684	2-chloroethyl 2,2-dichlorovinyl methyl phosphate	92	86	—	—	—	—
24869	1-chloro-2-propyl 2,2-dichlorovinyl ethyl phosphate	92	82	—	—	—	—
Dibrom®	1,2-dibromo-2,2-dichloroethyl dimethyl phosphate	82	90	82	38	—	—
Heyden Newport Newphos No. 1	1-methoxycarbonyl-2-buten-3-yl dimethyl phosphate	82	—	—	—	—	—
Gen. Chem. 3707	1,3-bis(methoxycarbonyl)-1-propen-2-yl dimethyl phosphate	82	—	—	—	—	—
Bayer 25820	diethyl naphthalimido phosphate	54	58	49	43	13	—
Schradan (Standard)		—	—	—	72	—	—
Malathion (Standard)		—	—	—	—	95	24

and Japanese beetles (*Popillia japonica* Newman) 6,250 g's for 2 minutes.

As a result of discussions on the effectiveness of impact and other types of forces involved in destroying the pink bollworm (*Pectinophora gossypiella* (Saunders)) in the ginning process, it became apparent that centrifugal and centripetal forces were also present. Therefore a study was undertaken to determine the effects of centrifugal force on pink bollworm larvae.

PROCEDURE.—Fourth-instar nondiapause larvae were collected from cotton blooms and mature green bolls. Only those larvae were collected that voluntarily emerged from bolls to make sure that the mature fourth-instar larvae were in the nondiapause state. Mature, fourth-instar diapause larvae, from infested seed cotton stored at approximately 15° C., were obtained by means of a Berlese funnel technique (Clark et al. 1959). A sample from each mature fourth-instar larval group was taken periodically to determine the pupation rate, and hence further determine the state of activity. Fourth-instar larvae treated were: An immature group; and four mature groups—nondiapause; and diapause stored less than 3 months, less than 5 months, and more than 9 months. In addition, fourth-instar larvae stored for 5 months and still in the seeds were treated to determine if the seeds offered any protecton. After treatment, the immature group of fourth-instar larvae was placed on a cottonseed-meal diet (Clark et al. 1960).

Larvae were centrifuged in either a large floor-model centrifuge equipped with both a timer and a variable-speed motor or a variable high-speed microcentrifuge. One larva was placed in each cup (four cups were available in the large centrifuge and eight in the microcentrifuge).

Mature, fourth-instar larvae, with the exception of larvae in seed, were subjected to relative centrifugal forces (R.C.F.) of 50 to 24,200 g's for periods of 1, 3, and 5 minutes. The time intervals did not include the acceleration and deceleration periods which, at the highest speeds, averaged 7 and 10 seconds, respectively, in the large centrifuge and 20 and 90 seconds in the microcentrifuge. Immature and mature fourth-instar larvae in cottonseed were subjected to forces of 50 to 2,500 g's for 1 minute. From 4 to 12 replicates with 4 to 8 worms per replicate were treated.

After treatment the larvae were placed in individual vials and kept in the laboratory at 29° C. After a careful 4-hour observation for immediate injuries, further observations were made at 24- or 48-hour intervals. Mortality was determined at the end of 10 days since preliminary tests showed that it took this long for all lethal effects to appear. The larvae were periodically examined after the 10-day period to determine if pupation and emergence rates, and resulting pupae and adults were normal. The median lethal R.C.F. (LG^{50}) was calculated from the computed regression-line formula.

RESULTS.—The lethal effects against mature fourth-instar larvae are shown in table 1. There was no mortality and severe injuries did not occur at forces below 2,500 g's for 1 minute for any of the larvae, including those inside cottonseeds. Some larvae were injured or crushed by forces of at least 10,000 g's and were occasionally eviscerated at 24,000 g's. One hundred per cent mortality was reached only at the R.C.F. of 24,200 g's in nondiapause larvae. Immediately after centrifugation, larvae surviving the treatments were quite restless, but usually within 2 hours they became relatively inactive and commenced to spin cocoons. No abnormalities in the pupae or adults from surviving larvae were observed.

Table 1.—The lethal effects of centrifugal force on fourth-instar pink bollworm larvae.

TIME OF APPLIED FORCE (MINUTES)	LG_{50} (GRAMS)	
	Nondiapause Larvae	Diapause Larvae
1	14,711	17,798
3	8,428	12,841
5	6,774	12,598

Among many of the nondiapause larvae subjected to the greater forces, a gradual darkening occurred, which masked the integumental pigmentation and indicated internal injuries. The pupation rate of the nondiapause larvae was within normal limits. A few of the immature fourth-instar larvae subjected to forces below 2,500 g's pupated, which brought about adult lengths of as little as 3 mm. (average length for the species is about 10 mm.).

A few diapause larvae appeared unoriented immediately after treatment but otherwise were not visibly affected by the centrifugal forces. Diapause was not terminated. The pupation rate of the treated larvae more than 3 months old was slightly faster than that of the control group.

DISCUSSION.—The slightest mass, such as a tuft of cotton resting on the larvae, was detrimental at the greater forces. Larvae were crushed if a ball of cotton was placed over them, which can be explained by the amount of force exerted on the larvae by the cotton. Leonard[4] found that a gram force of 367.7 ±73.5 would crush mature pink bollworm larvae and this force could be achieved easily by approximately 3,700 g's with a 0.1-gram ball of cotton.

An R.C.F. of approximately 13,800 g's would place the larvae under a force of 368 grams, which explains the destruction of some larvae at R.C.F.'s above 10,000 g's. Diapause larvae, with an average weight of 26.7 mg., would be under a force of 646 grams at 24,200 g's (the highest R.C.F. used).

A linear line was obtained when per cent mortality (Y) and log (R.C.F.×time) (X) were graphed; $Y=47.10X-146.07$ for nondiapause larvae and $Y=49.99X-173.79$ for diapause larvae. Thus, within the limits of these experiments, larvae subjected to a lower centrifugal force for a longer time interval would react much the same as those subjected to a higher force for a shorter time.

The phenomenon of forcing pupation in immature nondiapause fourth-instar larvae is probably an example of a survival reaction to physical damage that otherwise would be lethal. The authors have noted this phenomenon in several species of lepidopterous larvae in which haemolymph was extracted to the point of exsanguination.

From the results of this study it was concluded that centrifugal force has no part in the destruction of pink bollworm in the ginning process. This study further confirmed the conclusion made by Sullivan & McCauley (1960) that insects can easily withstand acceleration forces developed in space travel.

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Heptachlor and Heptachlor Epoxide Residues in Truck Crops[1]

L. H. DAWSEY,[2] D. W. WOODHAM,[2] and C. S. LOFGREN,[3,4]
U. S. Department of Agriculture

In 1960 a study was made in southern Mississippi to determine if residues of heptachlor or heptachlor epoxide would occur in truck crops grown in soil treated with ¼ or ½ pound of heptachlor per acre, applied as a granular formulation.

TREATMENTS.—The area selected for tests was near Wiggins, Mississippi, in a sandy loam type of soil. Three plots of about 1 acre each were used. One plot was treated with 10 pounds of 2½% heptachlor granules (¼ pound per acre), another with 10 pounds of 5% heptachlor granules (½ pound per acre), and the third was the untreated check. The applications were made with a Gandy fertilizer distributor on March 17. Twenty-eight days later, the plots were prepared for planting according to normal farming procedures. On April 15, the plots were fertilized with a commercial grade 6-8-8 fertilizer at the rate of 1,200 pounds per acre. All crops were planted on April 19 and 20, except sweetpotatoes, which were planted on June 13. Two 100-foot rows of each crop were planted in the treated plots and two 200-foot rows in the check plot. Normal cultivation procedures were followed after planting and no other insecticides were applied.

SAMPLING.—The crops were harvested as they matured—radishes in May, sweetpotatoes in October, and the others at intermediate dates. The samples were shipped, as harvested, to the analytical laboratory at Tifton, Georgia, in three separate polyethylene bags. The first contained crops from an untreated plot, the second from the ¼-pound treated plot, and the third from the ½-pound treated plot.

ANALYTICAL METHODS.—Each sample was separately analyzed for heptachlor and heptachlor epoxide by the methods developed by Ordas et al. (1956) and Meyer et al. (1960), respectively. Produce was prepared prior to analysis by cleaning as it would have been for kitchen use, that is, by washing off soil, or removing the outer leaves from cabbage, lettuce, onions, and so forth. A 200- to 1,000-gram aliquot of the edible part was chopped in a vegetable chopper with about twice the weight of anhydrous sodium sulfate, and the mixture transferred to gallon glass jars and tumbled with 500 ml. of colorimetric hexane to extract the insecticide. The decanted hexane extract was filtered, aliquoted and evaporated to dryness at room temperature with an air jet. The residues were then taken up in colorimetric pentane and chromatographed through a Florex column to separate the heptachlor from the epoxide. Extracts of carrots, onions, turnip roots, turnip tops, peanuts, and sweetpotatoes, which contained oils or other interfering substances, were treated with fuming sulfuric acid (5% sulfur trioxide) prior to passage through the chromatographic column. The first elution from the column, consisting of 200 ml. of pentane, contained the heptachlor. This elution was followed by 200 ml. of pentane containing 5% ether to elute the epoxide. Colors were developed by the recommended procedures and absorption was measured in a 0.5-ml. cell with a 1-cm. light path.

The samples were analyzed in duplicate. Each crop was thus carried through the procedure in sets of six samples at a time: Two controls, two treated at ¼ pound, and two at ½ pound.

When control samples of untreated crops were carried through the procedure, all showed appreciable absorptions. The quantity of insecticides present in a treated sample was obtained by deducting the two absorption readings of the controls from the reading of the treated sample. The micrograms of insecticide corresponding to the differences in absorption between each treated sample and the two controls, taken separately, were read on a zeroed standard curve prepared beforehand. Two residue values were then calculated. The residue found in a treated sample was expressed as a range, rather than as a single figure, which might have implied a higher degree of experimental accuracy than was warranted.

Table 1.—Residues found in truck crops grown in soils treated with granular heptachlor at Gulfport, Miss., 1960.

VEGETABLE	TYPE OF RESIDUE[a]	RESIDUE[b] RECOVERY (%)	RESIDUES (P.P.M.)[c] FROM HEPTACHLOR APPLIED TO SOIL AT 0.25 Lb./A.	RESIDUES (P.P.M.)[c] FROM HEPTACHLOR APPLIED TO SOIL AT 0.50 Lb./A.
Beans, green pods	H	105	0.13–0.13[d]	0.00–0.01
	E	100	.01– .01	.01– .01
Cabbage heads	H	140	.00– .00[e]	.02– .03
	E	105	.02– .01[e]	.00– .02
Cantaloup, meat	H	80–105	.00– .00	.00– .00
	E	190–195	.00– .00	.00– .00
Carrot, roots	H	73–78	No samples	.02– .03
	E	97–107	— —	.00– .03[e]
Cucumbers, whole	H	95–110	.00– .00	.02– .02
	E	98–102	.00– .00	.00– .01
Lettuce, heads	H	110	.07– .10[e]	.00– .00
	E	100–115	.00– .00[e]	.00– .00
Okra, pods	H	105	.00– .00	.00– .00
	E	107–115	.00– .01	.00– .01
Onion, bulbs	H	70–78	.05– .07[d]	.00– .01
	E	95–101	.03– .04	.03– .04
Peanuts, green pods	H	90–114	.02– .02	.02– .02
	E	138–172	.00– .01	.00– .00
Peas, green pods	H	117–122	.00– .00[e]	.00– .00
	E	93–98	.00– .00[e]	.00– .00
Potatoes, Irish	H	95–96	.01– .01	.01– .01
	E	95–103	.00—.01	.01– .02
Radish, bulbs	H	120	.00– .00	.01– .01[f]
	E	112–120	.13– .13	.11– .15[f]
Sweetpotatoes,	H	100	.01– .02	.00– .01
	E	100	.00– .00	.00– .00
Turnip, green tops	H	115–118	.00– .00	.00– .00
	E	65–72	.00– .01	.00– .00
Turnip, roots	H	—	.02– .02	.00– .00
	E	75–78	.00– .00	.00– .00
Watermelon, meat	H	110–112	.00– .01	.01– .02
	E	115	.00– .00	.00– .00

[a] H = heptachlor, E = heptachlor epoxide.
[b] When the two unfortified control determinations were not the same, recovery is expressed as a range; but when identical, recovery is reduced to a single figure.
[c] Unless otherwise indicated, range of analysis is the mean from duplicate determinations obtained against two control determinations taken separately.
[d] One of the duplicate samples considered contaminated from an unknown outside source.
[e] Figure from a single sample.
[f] Figures obtained from three samples.

Recovery tests for each compound were made separately for the various crops before analyses of treated samples were undertaken by carrying one control sample fortified with 50 μg. of insecticide through the entire procedure against two unfortified controls. The ranges so established are shown in table 1. Heptachlor recoveries ranged from 70% to 140% depending upon the crop and variation in controls, whereas the epoxide ranged from 65% for turnip tops to 195% for cantaloup meat. The apparent high epoxide recovery from cantaloup meat was owing to the omission of the acid cleanup step which should have been included. However, no residues were later found in the treated cantaloup samples when analyzed without acid cleanup.

Table 1 shows the data obtained in analysis for heptachlor and heptachlor epoxide in 16 different truck crops. Proper interpretation of the data requires definition of what constituted a detectable residue. The lower limit to which residues were measurable

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[2] Entomology Research Division, Agric. Res. Serv., Tifton, Georgia.
[3] Plant Pest Control Division, Agric. Res. Serv., Gulfport, Miss.
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in the present experiment was taken arbitrarily to be about 0.03 p.p.m., and no set of analyzed samples was considered to have shown a detectable residue unless the amount exceeded or equalled 0.03 p.p.m. of insecticide. No residues were detected in 14 of the 16 crops tested, but the radishes and onions showed significant amounts when grown in plots treated with either ¼ or ½ pound of heptachlor per acre.

The onions contained from 0.03 to 0.08 p.p.m. and the radishes from 0.13 to 0.20 p.p.m. of total residue. Other root crops analyzed but not showing detectable residues included carrots, peanuts, potatoes, and turnips.

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Increasing the Rate of Egg Productivity in *Aedes aegypti*[1]

ROBERT E. WILLIAMS and DWIGHT M. DELONG,
The Ohio State University, Columbus

In a study of the effect on egg deposition of the addition of color to the oviposition medium (Williams, unpublished) found that *Aedes triseriatus* (Say) deposited more eggs to a colored oviposition medium than to clear water. A single test using *Aedes aegypti* (L.) showed that it deposited more eggs to a colored medium also (a 3 to 1 ratio).

An experiment was then arranged to investigate whether *Aedes aegypti* would deposit more eggs per unit of time if colored water were used as an oviposition medium. In short, the color was to serve as a catalyst.

The color substance used was Burnett's Food Color. Its contents are listed as propylene glycol, tartaric acid, and U. S. Certified food color. It is not known whether the increase in egg productivity was caused by some effect of one of the constituents of the dye, or to a visual response to the color.

METHODS AND PROCEDURES.—Twenty cages, containing 10 females and 20 males each, were placed in a room maintained at 80±5% relative humidity and 80±2° F. They received 12 hours of fluorescent illumination at 26 foot candles plus a 1-hour simulated twilight period in the morning and evening each day. They were fed a 10% sucrose sulution on a gauze pad which was replaced every third day. The females were given a blood meal (laboratory mouse) every fourth day. In one series of 10 cages, 150 ml. of tap water in a plastic cup with paper toweling as an oviposition surface served as the oviposition device for each cage. In the second series of 10 cages, the same arrangement was used except that a dark brown color was produced in the oviposition water by adding two ml. each of red and green food color to 2 liters of tap water. This was then poured in 150-ml. aliquots into plastic cups. The cages containing the two types of oviposition media were placed in alternate order on the holding shelves.

The test adults emerged from one group of larvae reared on dried bovine blood. The mosquitos were permitted to emerge into a single large cage which was then placed in a walk-in cooler (45° F.). After chilling they were counted out at random, placed in the test cages, and the cages moved to the holding room. The test was carried 30 days from the first blood meal. Mortality was

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Table 1.—Eggs deposited in colored-water and in clear-water cups.

Deposition Periods	Replicates 1	2	3	4	5	6	7	8	9	10	Totals
Deposited in colored-water cups											
1	201	511	525	508	600	651	289	314	672	434	4,705
2	0	49	0	0	0	0	0	0	0	0	49
3	57	206	399	301	318	196	162	164	236	427	2,466
4	530	446	561	504	616	372	167	458	562	565	4,781
5	0	0	0	0	0	0	0	0	0	0	0
6	482	785	726	777	809	547	691	786	794	726	7,123
7	0	0	0	0	0	0	133	0	0	0	133
8	653	463	529	706	320	405	266	559	282	212	4,395
9	171	227	461	258	129	248	204	288	556	434	2,976
10	0	0	16	0	0	0	94	16	0	0	126
Totals	2,094	2,687	3,217	3,054	2,792	2,419	2,006	2,585	3,102	2,798	
Grand total											26,754
Deposited in clear-water cups											
1	351	316	714	518	397	663	472	226	743	371	4,777
2	0	0	0	23	0	0	0	0	45	38	106
3	207	104	301	186	321	58	136	318	318	311	2,260
4	366	86	481	432	384	66	73	344	235	180	2,647
5	0	0	0	0	0	0	0	0	0	0	0
6	265	318	735	708	375	731	252	562	740	455	5,161
7	0	0	0	84	0	0	0	0	0	108	192
8	294	205	499	199	495	382	133	95	419	118	2,839
9	118	181	140	316	544	351	470	548	271	450	3,389
10	0	0	0	81	0	63	0	27	0	0	171
Totals	1,607	1,210	2,890	2,547	2,516	2,314	1,536	2,120	2,771	2,031	
Grand total											21,542

Table 1.—Mortality of salt-marsh caterpillars sprayed with 4 pounds of insecticide per 100 gallons and held without food.

Insecticide and Formulation	Chemical Name (if proprietary product)	Percentage Mortality After— 24 Hours	48 Hours
	Experiment 1		
Phosdrin® EC	1-methoxycarbonyl-1-propen-2-yl dimethyl phosphate	97	100
Sevin® WP	1-naphthyl *N*-methylcarbamate	96	99
Dibrom® EC	1,2-dibromo-2,2-dichloroethyl dimethyl phosphate	94	98
Parathion WP	—	77	95
Diazinon® WP	*O,O*-diethyl *O*-(2-isopropyl-6-methyl-4-pyrimidinyl) phosphorothioate	47	85
Dilan EC	1 part of 1,1-bis(*p*-chlorophenyl)-2-nitropropane plus 2 parts of 1,1-bis (*p*-chlorophenyl)-2-nitrobutane	47	76
Phorate EC	—	28	53
Demeton EC	—	13	34
Thiodan® WP	6,7,8,9,10,10-hexachloro-1,5,5*a*,6,9,9*a*-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide	9	33
Dicapthon WP	—	20	32
Trithion® EC	S-(p-chlorophenylthio)methyl *O,O*-diethyl phosphorodithioate	14	31
Endrin EC	—	17	29
Malathion WP	—	7	27
Heptachlor EC	—	11	26
Toxaphene WP	—	5	20
Untreated	—	0	2
L.S.D. at 5% level		13	16
	Experiment 2		
Union Carbide 10854	*m*-isopropylphenyl methylcarbamate	97	100
Sindar Corp. A-1634	butyl 1,3-dimethylbutyl acetal of acetaldehyde	95	99
Bayer 24,869	1-chloro-2-propyl 2,2-dichlorobinyl ethyl phosphate	93	98
Parathion WP	—	77	97
Ent. 21, 195	6-bromopiperonal chrysanthemumate	82	95
Gen. Chem. 3707	1,3-bis(methoxycarbonyl)-1-propen-2-yl dimethyl phosphate	89	94
Hercules 3895	2,2-bis(ethylthio)vinyl diethyl phosphate	52	80
Bayer 25,141	*O,O*-diethyl *O*-(*p*-methylsulfinyl)-phenyl phosphorothioate	48	66
Gen. Chem. 3661	1,3-bis(methoxycarbonyl)-1-propen-2-yl diethyl phosphate	36	57
Bayer 25,198	*O,O*-dimethyl *O*-p-methylsulfinyl-phenyl phosphorothioate	36	52
Guthion® WP	*O,O*-dimethyl *S*-(4-oxo-1,2,3-benzotriazin-3-(4H)-ylmethyl)phosphorodithioate	27	51
Hercules 3653	diethyl 2-methylthio-1-methylvinyl phosphate	26	45
Hercules 3004	diethyl 1-ethylthio-1-propen-2-yl phosphate	25	41
Bayer 25,453	*S*-2-(ethylsulfinyl)ethyl *O,O*-dimethyl phosphorodithioate	23	40
Bayer 22,893	*O,O*-diethyl *O*-1,2,2-trimethylpropyl phosphorothioate	19	36
Bayer 16,259	*O,O*-diethyl *S*-(4-oxo-1,2,3-benzotriazin-3-(4H)-ylmethyl) phosphorodithioate	18	36
Untreated		0	1
L.S.D. at 5% level		13	14
	Experiment 3		
Gen. Chem. 3583	*O,O*-diethyl-1-(2,5-dichlorophenyl)-2-chlorovinyl diethyl phosphate	86	100
Gen. Chem. 3582	*O,O*-diethyl-1-(2,5-dichlorophenyl)-2,2-dichlorovinyl phosphate	86	93
Gen. Chem. 4072	*O,O*-diethyl-1-(2,4-dichlorophenyl)-2-chlorovinyl diethyl phosphate	67	92
Bayer 22,408	*O,O*-diethyl *O*-naphthalimido phosphorothioate	17	55
Monsanto CP-7769	hexaethyl (ethylthiomethylidyne) triphosphonate	15	50
Monsanto CP-12,432	diethyl 1-(dimethoxyphosphinyl) vinyl phosphate	15	30
Untreated		1	5
L.S.D. at 5% level		13	13

recorded every other day. Egg cups were replaced every third day.

RESULTS.—The 10-cage series receiving the colored water as the only significant variable deposited a total of 26,754 eggs, to 21,542 eggs for the 10-cage series with tap water as the oviposition medium (see table 1). This is an increase of 5,212 eggs or 24% more than the clear water series. Inspection of table 1 shows that the increase occurred generally throughout all replications of the series and did not come about sporadically, with excesses in one or two replicates. Moreover, more eggs were deposited to the dark-water cups with fewer females ovipositing since there was a higher mortality in this group until the last 3 days of the test. The clear-water series had one to three more females until the last 3 days when the colored-water series had three more than the former. There was also a slight but insignificant increase in per cent hatch in the dark-water series.

Laboratory Tests of Insecticides against the Salt-Marsh Caterpillar[1]

F. H. Harries, *Entomology Research Division, Agric. Res. Serv., U. S. D. A.*

The salt-marsh caterpillar (*Estigmene acrea* (Drury)) often occurs in enormous numbers in Arizona on cotton in the fall. As the cotton crop approaches maturity, the large caterpillars migrate from cotton to vegetables and are very difficult to control with insecticides. Increasing resistance to some insecticides has been reported by Stevenson et al. (1957) and Wene et al. (1960). Some of the more important insecticides and a number of promising new compounds were compared in laboratory tests to find if any of these were sufficiently toxic by contact to be effective against the large migrating caterpillars. The tests were made in Phoenix, Ariz., in 1957.

Large migrating caterpillars were brought to the laboratory and counted into cylindrical 1-pint cartons that were capped with netting to minimize any fumigation effects of the insecticides. The sprays were all applied at 4 pounds of the actual material per 100 gallons with a small atomizer and a bicycle-pump compressor that delivered a constant amount of spray each time the treadle was depressed and then released. The larvae were shaken to the bottom of the carton and after the lid was removed were treated by one stroke of the pump or with about 2 ml. of spray that was directed downward from a height of 6 inches. The cartons were recapped and held on a laboratory table at 75° to 80° F. Examinations were made after 24 and 48 hours to record the mortality. No food plants were used. Ten larvae were treated in each carton and the tests were replicated 10 times on different days. New cartons were used for each test.

Results of the tests are all given in table 1. In the first experiment, data for the 24-hour observations show that Phosdrin,® Sevin,® and Dibrom® were significantly more toxic than any of the other materials. The results after 48 hours show that these materials were significantly more toxic than all of the other compounds except Diazinon.® In these tests, with the single exception of malathion, the phosphorus compounds were more toxic than the chlorinated hydrocarbon materials. It is suggested that some acquired resistance to toxaphene-DDT mixtures may have resulted in cross-resistance to heptachlor and endrin. Results of experiment 2 show that several of the new materials compared favorably with parathion in toxicity and that the first six materials as listed were significantly more toxic than the last 10 compounds. In the third experiment, three more compounds were very toxic and significantly better than the other three materials tested.

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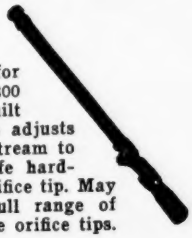

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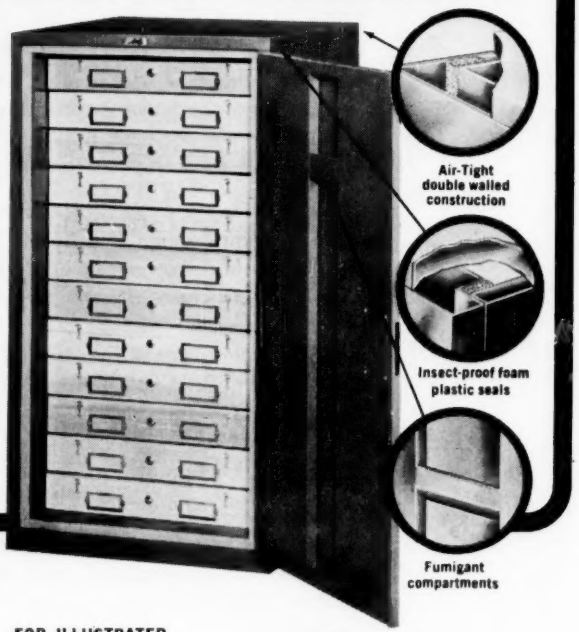

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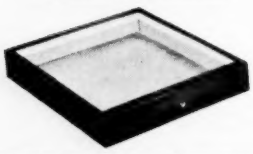

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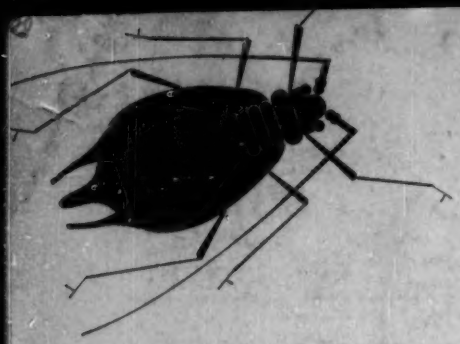

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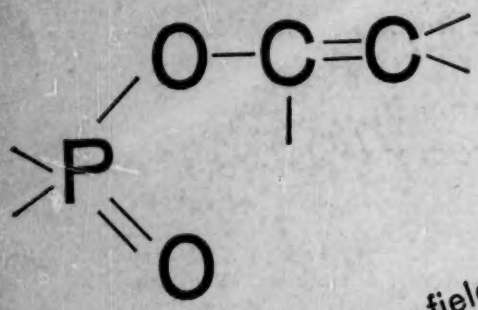

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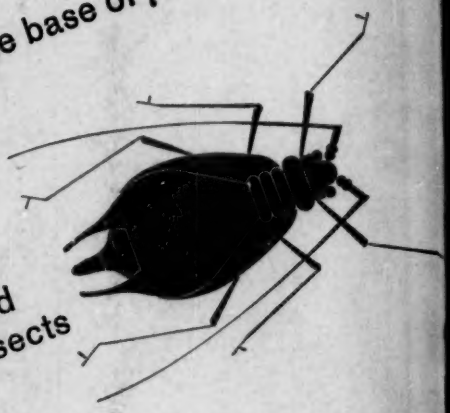

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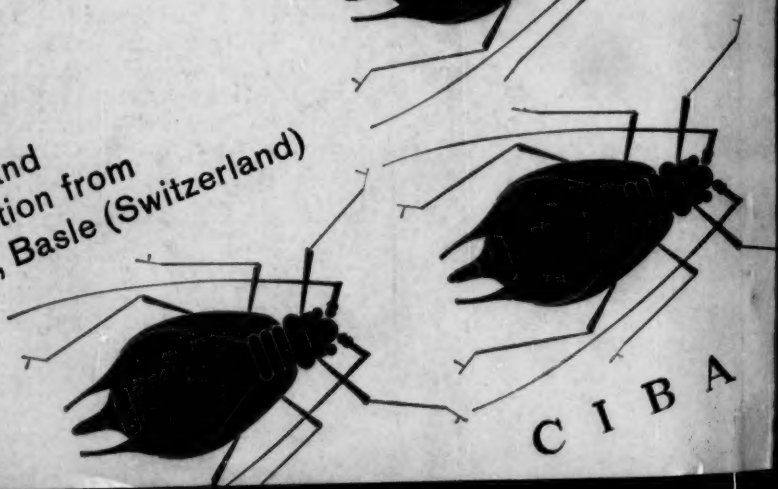

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